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COPPER CONTENT OF CITRUS LEAVES AND FRUIT IN RELATION TO EXANTHEMA AND FUMIGATION INJURY[1, 2]

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INTRODUCTION

A STUDY OF THE COPPER CONTENT of citrus leaves and fruit is of importance for four reasons: (1) The essential need of copper for certain plants is well known, but there is no knowledge regarding the copper content in citrus. (2) Methods frequently used to cure exanthema are the application of copper sulfate to the soil, or better still, the spraying of the entire tree with bordeaux mixture. Protection against brown rot disease is secured by spraying the lower portions of trees with bordeaux. (3) Increased and prolonged injury sometimes results when citrus trees are fumigated too soon after the application of copper. (4) An investigation of the copper content of citrus may throw some light on the cause of exanthema. This paper is concerned with the copper content of citrus in relation to these factors.

PREVIOUS WORK

It was shown by Sommer(37) that as little as 0.06 mg of copper per liter gave normal growth of sunflowers, tomatoes, and flax, as compared with very limited growth or death of the plant in culture solutions containing no copper. A deficiency of copper was not found to produce chlorosis. However, it was found by Anderssen(2) that chlorosis of deciduous fruit trees was due to a copper deficiency and was cured by the use of copper applied to the soil or leaves. Lipman and MacKinney(25) found the

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essential need for copper by flax and barley to be primarily concerned with the ability of the plant to produce seed.

According to Allison, Bryan, and Hunter,[1] copper sulfate and other chemicals stimulated plant response on the raw peat soils of the Florida Everglades, although the mode of action was little understood. Cook,[5] in experiments on respiration, has shown that copper penetrates *Nitella* and *Valonia* cells almost immediately. When copper chloride was allowed to act on *Aspergillus niger*, there was an interval of no change, called the latent period, followed by a fall in the rate of respiration.

The function of copper in plants is so little understood that it is necessary to follow the progress of studies with copper in animals. The inability of metals other than copper to supplement iron in curing nutritutional anemia of rats was emphasized by Underhill, Orten, and Lewis[41] as well as by Waddell, Steenbock, and Hart.[42] The specificity of copper as a supplement to iron has received very little support from the results obtained by Myers and Beard,[28] or by Titus, Cave, and Hughes,[40] who found a group of substances rather than a single substance active in hemoglobin building. It is of interest that Elvehjem, Steenbock, and Hart[9] have concluded that copper is not a constituent of the hemoglobin molecule in rat blood.

According to Elvehjem,[7] the beneficial action of copper is not due to its functioning in the assimilation of iron, but rather to its conversion of the iron into forms which can be used for the construction of the hemoglobin molecule. The nonhemoglobin or tissue iron is considered by Josephs[21] as being divided into a mobile and a fixed portion. Copper influences the mobile portion in two ways: it prevents the cells that ordinarily store iron from absorbing it or causes them to give it up; or it increases the rate of hemoglobin formation by a catalytic action or decreases the rate of hemoglobin breakdown. Cunningham[6] reported that copper feeding of rats converted inorganic to organic iron. These results obtained by feeding copper to animals serve to indicate the complexity of the role of copper in animal metabolism. Furthermore, both plant and animal nutrition investigators are aware of the minuteness of the amounts of copper that are necessary as catalysts in the normal metabolism.

The essential need of citrus for copper is of importance not only in itself, but also because of the relation of this need to the occurrence and cure of exanthema. The cause of exanthema, while considered by investigators (see Fawcett and Lee[11] and Rhoads and DeBusk[33]) to be one of malnutrition, may be due to some obscure organism or to the absorption or formation of a toxin by the tree.

Floyd[12] and later Stevens,[38] concluded that organic sources of

nitrogen were related to the occurrence of the disease in Florida. Fudge[13] found the quantity of nitrogen in the twigs of diseased trees to be three times that in healthy twigs, but found no differences in the fruit juices. Ruprecht, Camp, *et al.*[34] reported an increase in all forms of nitrogen, a decrease in iron, and an increase in phosphoric acid in leaves from diseased trees. Their results resemble the analyses of mottled leaves by Kelley and Cummins[22] and by Haas.[16] In advanced stages of exanthema the leaves may become severely mottled. Investigations are now under way to determine the role of copper in mottle-leaf.

The theory of poor nitrifying power of soils with consequent enforced ammonia absorption by the roots as a possible cause of exanthema and mottle-leaf, was advanced by Lipman.[24] However, exanthema-affected and mottled trees are not necessarily deficient in total nitrogen. Mottle-leaf usually occurs under conditions of soil fertilization with nitrogen from any source, regardless of the nitrifying power of the soil. Mottle-leaf was readily induced in soil or sand cultures (Haas[16]) by the use of nitrogenous compounds of the urea or amino type with the enforced absorption of these poisonous compounds. Nothing is known regarding the relation of copper to such compounds in the soil or tree.

Recently, Oserkowsky and Thomas,[30] in studies on exanthema in pears, found practically no difference in the copper content of diseased and healthy-appearing leaves from diseased trees, but found nearly twice as much copper in healthy leaves from part of the orchard free of the disease. Leaves from orchards in localities free of the disease contained about three to four times the amount of copper found in diseased leaves. The suggestion was made that exanthema in pear trees is due to a deficiency of copper. The mode of action is assumed to be the neutralization of soil toxins or the correction of a deficiency of copper.

Smith and Thomas[36] reported the beneficial effects of copper sulfate when applied to the soil. Thomas[39] found that the addition of copper sulfate to heavy soils was at first ineffective, but later was beneficial, and that tree injection with copper sulfate was effective. McCleery and Stokes[26] were successful in curing exanthema of citrus by means of copper sulfate applications to the soil or by spraying the top with bordeaux.

It is of interest (Allison, Bryan, and Hunter[1]) that although copper sulfate gave outstanding results with plants on the Florida Everglades soil, other chemicals, such as manganese, caustic lime, or manure, also brought about considerable improvement. Orth, Wickwire, and Burge[29] state that copper sulfate has come to be used very extensively by Florida orange growers as a remedy for "frenching." Accordingly, they treated soil with copper sulfate and found that the leaves of treated

trees contained 4.6 times more chlorophyl than the leaves of the control trees. They concluded from this experiment that copper is necessary for chlorophyl formation.

It should be pointed out in this connection that the application of copper sulfate to the soil has long been recognized in Florida and California in the United States, and on other continents, as a remedy for exanthema, but that no one previous to Orth, Wickwire, and Burge[29] has claimed copper sulfate as a cure for frenching (cf. Fawcett and Lee[11] and Rhoads and DeBusk[33]). In fact in the early stages of exanthema, citrus leaves may be unusually green and become mottled or chlorotic generally only after a prolonged attack of exanthema. In curing such advanced stages of exanthema, therefore, with copper sulfate, mottle-leaf or chlorosis may be overcome as a stage in the disease. Ordinarily, mottle-leaf is not considered a stage of exanthema. Whether mottled leaves are deficient in copper is a question which is now being studied.

Haas[17] was able to produce some of the symptoms of exanthema in citrus by the omission of manganese from the culture solution. In these studies, copper was not added to the culture solution; therefore, it is possible that a deficiency of copper brought about the exanthema symptoms. Experiments reported later in this paper show that this was probably the case.

It is also conceivable that iron compounds in hardpan formations which commonly underlie trees affected with exanthema may be able to precipitate the copper and manganese from the soil solution. Thus, the benefits obtained when such hardpans are broken up may be due to the continuance of an available supply of copper or manganese. The relative ease of curing exanthema in citrus with copper sulfate has prevented the use of a wide range of chemicals in attempting to cure the disease, and as a consequence very little light has been shed on the causal factors. In the case of exanthema of pears, however, zinc, manganese, and iron salts, respectively, have been found by Oserkowsky and Thomas[30] to be without effect on the disease.

Citrus growers, however, in districts where fumigation is practiced for scale control, have recently called attention to the need of cures for exanthema, other than by the use of copper sulfate. The fact that an increased amount of copper in soil or trees may bring about severe injury when fumigated with HCN was one of the reasons for determining the copper in citrus under different conditions.

Fawcett,[10] Guba,[14] Butler and Jenkins,[4] and Woglum[46] call attention to the effects of cyanide fumigation on plants following spraying with bordeaux mixture. Guba and Holland[15] attributed the injury

to plants sprayed with bordeaux mixture prior to fumigation as being due to the formation of soluble copper on the foliage. In the absence of fumigation of such sprayed plants, no soluble copper was found in the water extract of the dry spray residues.

Suggestions regarding bordeaux mixture and its physiological action were published by Wilson and Runnels,[44] who found with *Coleus* plants that the comparative transpiration rates of treated and untreated plants vary widely during different portions of the 24-hour period. The treated lose much more water than the untreated plants during periods of darkness. Since fumigation is practiced during the night, the greater movement of water in the treated plants at that time may bring about an increased solubility of HCN in the tissue fluids, with resulting greater injury as a consequence of intracellular acidity, cell asphyxiation, or other reactions.

In a later paper, Wilson and Runnels[45] reported that, although bordeaux mixture increased the water requirement of *Coleus,* tomato, and cucumber plants grown in soil kept at definite moisture contents, the combining of an oil emulsion (which when used alone decreased the transpiration rate) with the bordeaux mixture, affected the water requirement and transpiration rate but little.

In many instances the use of copper in combating citrus diseases where fumigation is practiced has led to severe injury to the trees. It would appear that copper used for such purposes is noninjurious when fumigated after an interval of three or more years, especially when the diseased trees have fully recovered. Growers frequently prefer to allow the diseased trees to go untreated unless they are assured that the use of copper followed by fumigation will not undo the good that the copper has done. At present, the injury is attributed to an increased copper content of the tree merely because copper was applied, but no data as to the increase are available. It is possible that citrus trees that consistently show a greater degree of injury to HCN fumigation than others may be found to contain a greater content of copper. Haas,[18] as a result of analyses of various scale insects, ventures to suggest that red scale insects containing the least copper may be the most resistant to HCN fumigation. The purpose of the copper analyses of citrus reported in this paper is to give some basis for the consideration of problems which at present have few chemical data.

SOURCE AND PREPARATION OF MATERIAL

Chemical determinations were confined to the leaves and fruit. The marked effect of exanthema on the fruit peel, with or without the occurrence of gum pockets in the angles of the pulp segments near the core,

made it desirable in most cases to separate the peel from the pulp. Because of the variability in the occurrence and number of seed in the fruit of the same or different varieties, it seemed desirable to discard the seed.

It was relatively easy to distinguish the diseased from the healthy fruit by the appearance of the peel and by a cross section of the pulp. The distinction between the leaves of healthy and diseased trees was often not an easy matter, even after the nature of the fruit and twigs had been taken into consideration. Consequently, healthy leaves were considered to be those taken from trees showing no twig or fruit symptoms, while diseased leaves were those taken from trees the twigs or fruit of which showed obvious symptoms of the disease.

The samples of mature citrus leaves were carefully wiped free of dust, dried at 80° C, and finely pulverized. Fruit samples were freed of the button, wiped clean, and in most cases the peel and pulp were dried separately. The pulp halves were placed in the oven at 80° C on clean glass plates or large sheets of filter paper, the cut surface of the pulp being uppermost. In this way, and by having the large, roomy oven not too full of fruit at any one time, it was possible to dry out the pulp sufficiently so that it could be broken up into fragments and dried further in paper bags until suitable for pulverization.

With the method used in the determination of copper, 75 to 100 grams of dry matter gave better results than smaller size samples. The leaf and fruit samples were collected in various locations in southern California where exanthema was found to occur.[5] Samples were also obtained from trees sprayed with bordeaux solution and from healthy and diseased trees growing in soil to which copper sulfate was applied.

DETERMINATION OF COPPER, IRON, AND PHOSPHORUS

Preliminary determinations of copper in citrus leaves and fruit were made by the method described by Ansbacher, Remington, and Culp,[(3)] using the chromotropic reagent. The results in some cases were extremely accurate, while in others they were erratic.

The method finally employed represented a combination of portions of methods described by others. It is described in detail in order to acquaint citrus associations and other laboratories with the procedure that was used. The samples of ground dry matter were burned in silica dishes with a low flame until much of the ash was white. Silica dishes (casseroles with a deep, wide mouth and with the handle cut off) of 75-cc capacity ($2\frac{3}{4}$ inches inside diameter $\times$ $1\frac{3}{8}$ inches deep) were used

[5] The writers are indebted to Mr. Harold E. Wahlberg, Farm Advisor, Orange County, for help in securing some of the samples.

for ashing the organic matter. Due to the bulk of the sample, several weighings were made on the analytical balance, and after the silica dish was filled, the remainder of the sample was transferred to glossy paper and was added to the dish as space was available. When the dishes were cool they were covered with watch glasses, and about 25 cc of 1:1 HCl were added. After digestion on the hot-plate the solution was filtered through a No. 40 Whatman filter paper into a 250-cc beaker. The cover glass, dish, and filter paper were then rinsed several times with hot water. The filter paper was transferred to the silica dish by means of a small piece of No. 40 Whatman filter paper. The dish was then dried on the hot-plate and the contents reignited until free of carbon. In most cases further ignition after filtration or the addition of nitric acid was unnecessary in order to remove all of the carbon.

After the second ignition, the dish was cooled and 25 cc of 1:1 HCl were added. After digestion under a cover glass on the hot-plate, filtration was again made through a No. 40 Whatman filter paper, using the original beaker and funnel. The dish was scrubbed with the addition of warm 1:1 HCl and thoroughly washed. The filter paper and funnel were then washed with hot water. The filter paper was folded and dried while the solution in the beaker was evaporated and the silica dehydrated in the usual manner.

When the beaker was cool, about 7.5 cc of concentrated HCl and some hot water were added, and the contents allowed to digest on the hot-plate. The solution was then filtered through a No. 40 Whatman filter paper into a 250-cc Erlenmeyer flask, and the precipitate carefully washed with hot water. The filter paper containing the silica, and that containing the sand and carbon, were both placed in a small platinum dish, dried, and ignited. When the dish was cool, a very small amount of water was carefully added in order to moisten and prevent the light ash from blowing away. A drop of concentrated H_2SO_4 was added and then sufficient HF to insure removal of the silica. The platinum dish was then heated on a piece of asbestos in the hood until dense, white SO_3 fumes were evident. Unless the amount of silica was large, a second evaporation with a drop of concentrated H_2SO_4 and some concentrated HF was unnecessary. When more than one drop of concentrated H_2SO_4 is used, there is danger of spattering when the evaporation is nearly complete.

When cool, about 5 cc of 1:1 HCl were added to the platinum dish, which was again heated on the asbestos in the hood until the ash was dissolved. Filtration through a No. 40 Whatman paper into the Erlenmeyer flask was followed by sufficient washing with hot water to bring the volume of solution in the Erlenmeyer to about 100 cc or a known

volume. If a 100 cc volume is used, then the solution contains approximately 10 per cent of HCl. The acid concentration should not exceed 15 per cent by volume.

It was found that more reliable results were obtained when the silica was removed. This is in harmony with the experience of Piper,[32] who found manganese to be more accurately determined after the removal of the silica.

HCl was used instead of H_2SO_4 because of the large amount of calcium present in citrus leaves and the consequent heavy precipitation of $CaSO_4$ when H_2SO_4 is used.

The flask was heated to boiling, and a current of hydrogen sulfide was allowed to flow through a capillary glass delivery tube inserted in one hole of a two-hole rubber stopper. The delivery tube was then placed in the flask containing the boiling solution. The flow of gas was continued until the solution was cold, usually about 15 minutes being required. By means of air suction the waste H_2S was removed from the bell jar containing the Erlenmeyer flask. The drop of concentrated H_2SO_4 used in the HF volatilization of silica assures sufficient sulfur in the solution, which helps to precipitate the copper sulfide.

When the solution was cold, the stopper and capillary tube were raised out of the Erlenmeyer and washed into the flask, while the gas was still flowing. The flask was closed at once with a rubber stopper and placed in a pan of cold water or otherwise kept cool overnight, after which time the supernatant solution was clear.

The solution was then filtered through a No. 42 Whatman filter into a 250-cc beaker. The empty flask was rinsed about five times with a special wash water kept at room temperature. This wash water was distilled water acidified with acetic acid (50 to 100 cc glacial acetic acid in a liter flask of water) and saturated with hydrogen sulfide under the bell jar. The back flow of hydrogen sulfide gas from the wash water was prevented in the following manner: a short piece of rubber tubing containing a Bunsen valve-cut was closed at one end with a glass rod and was attached to the lower end of the inlet tube of the wash bottle. Finally, the filter paper was rinsed about five times with this wash water. Such washing usually was sufficient to dissolve all traces of iron and carry it into the beaker. During the filtration care should be taken to keep the copper sulfide bathed as much as possible with the wash water containing H_2S to prevent changes in the nature of the precipitate, and possible solubility changes due to too prolonged contact with the air. The filter paper containing the copper sulfide was removed from the funnel by the use of a small piece of Whatman filter paper and transferred to the Erlenmeyer flask. The top of the funnel was scrubbed

with a small piece of the filter paper which was also added to the flask. The funnel was then rinsed with the wash water and the beaker set aside for the determination of iron.

About 10 cc or more of half concentrated HNO_3 were added to the flask, which was then heated on the hot-plate in order to macerate the filter paper; the heating was continued until brown fumes were evolved. This method was found to be as efficient and less troublesome than using fuming nitric acid at the outset. The contents of the flask were diluted with hot water to a volume of 25 cc or more; otherwise, in the subsequent filtration the acid disintegrates the filter paper.

The warm contents of the flask were filtered through a No. 42 Whatman filter, the apex of which was protected with a porous platinum or platinum-alloy cone. The funnel was placed through a rubber stopper in the top of a bell jar and the funnel stem in a 150-cc, or smaller, beaker. Suction was applied at the side outlet of the bell jar. Hot water was used in washing the flask and filter paper.

Another procedure in separating the copper and iron is as follows: When the volume of sulfur and copper sulfide precipitate in the Erlenmeyer flask is extremely small, the copper sulfide may be separated from the solution by the use of a porous-bottom crucible and the copper sulfide dissolved after the removal of the iron. The nature of the precipitates obtained with citrus leaf and fruit samples generally made it very wasteful of time to use the porous-bottom crucible, even though it saved one filtration. This was especially true when 75 to 100-gram samples of dry matter were used. Prior to being used for a second determination, such crucibles were improved by treatment with hot alkali followed by weak acid and hot water. The value of this method of cleaning the crucibles was increased when a furnace was available for the ignition and slow cooling of the crucibles, a procedure which prevented the cracking of the porous bottom.

Regardless of which of the two procedures was used for separating the copper from the solution, the subsequent procedure was as follows: The solution containing copper was evaporated barely to complete dryness on a hot-plate. Care should be taken to avoid decomposing the nitrate. Whitehead and Miller[43] have recently reported that it is not necessary to evaporate the mineral acids completely in the iodometric determination of copper, if a small amount of H_2SO_4 is present. Time is thereby saved and the danger of forming insoluble basic cupric salts obviated. This is in agreement with details of the iodometric method described by Scott.[35]

Dilute ammonia was added to the beaker and the solution heated on the hot-plate until the odor of ammonia was faint. Five cc of glacial

acetic acid were added to produce acidity to litmus, and the solution was boiled for about 1 minute and then cooled. Three to 4 grams of solid KI, or its equivalent, were added, and the solution was titrated with standard thiosulfate freshly prepared by diluting 20 cc of 0.1 N strength to 1 liter. One cc of this solution (0.0020N) had a copper value of 0.1271 milligram. Starch solution was added, and the titration was continued from a 10-cc microburette until the end-point was reached.

In the determination of copper in soil, 100 grams of air-dry soil were treated with a mixture of 80 cc of nitric acid and 20 cc of sulfuric acid in a liter beaker. The mixture was heated on the hot-plate until the nitric acid fumes were removed. Repeated additions of HCl were made, and the evaporation was continued in order to dehydrate the silica. The copper was dissolved with 1:1 HCl by digestion of the silica mass on the hot-plate. With the aid of suction, the silica was separated by filtration and was volatilized as previously described. Iron in the filtrate was precipitated with ammonia, filtered with suction, and redissolved with 1:1 HCl; it was then reprecipitated and removed by filtration. The combined filtrates from the silica and two iron filtrations were evaporated to about 100 cc and transferred to an Erlenmeyer flask. The HCl-soluble portion from the residue left after the silica volatilization was filtered into the flask and the acidity regulated to 10 per cent HCl. The previously described method was then used in the precipitation and determination of the copper.

Iron and phosphate (ash) were determined in the filtrate from the copper separation by the method of Elvehjem and Hart,[8] the phosphate being weighed as magnesium pyrophosphate. In some cases iron was separated by the use of cupferron solution as described by Hart.[20]

CHEMICAL DETERMINATIONS

The method for the determination of copper in citrus material permits of considerable accuracy. We may cite a typical case in which a 50-gram sample of pulverized dry-leaf tissue showed 4.70 parts per million of copper in the dry matter. Another 50-gram sample of the same lot of tissue to which 5 parts per million of copper were added, showed 9.68 parts per million of copper, which indicates almost complete recovery of the added copper.

Table 1 gives the copper and iron content of citrus leaves (samples 1, 2, and 3) collected by H. S. Fawcett and H. J. Quayle at Upland on November 9, 1933. The control orange and lemon leaves were taken from adjoining groves that had never been sprayed with bordeaux. The control lemon leaves showed nearly double the copper content of the control orange leaves. A marked increase was shown in the copper con-

TABLE 1

COPPER AND IRON CONTENT OF CITRUS LEAVES FROM TOPS OF TREES

Sample No.	Place and date of collection	Variety	Treatment*	In dry matter†	
				Cu	Fe
				p.p.m.	*p.p.m.*
1	Upland, Nov. 9, 1933	Orange	Control for sample 3	6.85	345
2	Upland, Nov. 9, 1933	Lemon	Control for sample 3	13.06	288
3	Upland, Nov. 9, 1933	Lemon	Base of trees sprayed each year for 20 years with bordeaux mixture	36.58	221
4	Hillcrest, near Corona, Jan. 10, 1933	Lemon (Lisbon)	Control	6.14	...
5	Near Hillcrest, Jan. 10, 1933	Lemon (Lisbon)	Base of trees sprayed with bordeaux mixture; tree tops injured by HCN	9.08	...

* Only lower portions of trees sprayed with bordeaux mixture; control trees never sprayed with bordeaux.

† Samples of dry matter ranged from 31 to 85 grams, with an average of about 60 grams.

TABLE 2

COPPER, IRON, AND ASH PHOSPHORUS OF MATURE GRAPEFRUIT LEAVES FROM TREES ON DEEP, SANDY SOIL AT INDIO, CALIFORNIA

Sample No.	Date of collection	Treatment	In dry matter*		
			Cu	Fe	PO_4
			p.p.m.	*p.p.m.*	*p.p.m.*
1	Sept. 28, 1932	Leaves from control trees in an adjacent grove that never received copper	6.40	165	...
2	Sept. 28, 1932	Leaves from a tree, the drip of which extended into a basin containing a Deglet Noor palm that received an application of 125 pounds copper sulfate on November 20, 1930. Tree appeared healthy, although palm was injured	7.40	111	...
3	May 16, 1933	Leaves from grapefruit trees in same grove as sample 2 but never received copper	7.38	200	1,810
4	May 16, 1933	Leaves from a healthy grapefruit tree injured to some extent by a single application of 75 pounds copper sulfate in an irrigation basin on December 15, 1931	9.22	153	1,840

* Samples of dry matter ranged from 32 to 50 grams.

tent of the lemon leaves taken from above the sprayed portion. This would indicate an absorption of copper by the leaves of the lower portion of the tree and its translocation to leaves higher up on the tree—assuming no increased root absorption, or the unavoidable contamination of these leaves during the spraying. The increased content of copper was associated with a reduced total iron content. The lower portions of these lemon trees have been sprayed with bordeaux mixture for 20 years without injury from HCN fumigation. Since this practice has been followed for so long a period without injury to the trees, there is much likelihood that in such locations the nature of the soil may also be a factor in the injury resulting from copper.

Sample 5, consisting of leaves from the upper portion of lemon trees injured by the use of HCN following the application of bordeaux mixture to the lower portion of the trees, showed an increased content of copper as compared with sample 4, consisting of leaves from the upper portion of lemon trees that never received bordeaux mixture and that never showed unusual HCN injury.

The soil from the control location[6] showed 14.25 parts per million of copper in the first foot, and 13.26 parts per million in the second foot; while in the location where bordeaux mixture was used, the first foot contained 14.98 parts per million and the second foot 12.75 parts per million. Samples of 100 grams of air-dried, sieved soil were used in each case. The accumulation of copper in the first foot of soil under trees that received bordeaux mixture was almost negligible.

The data in table 2 are of interest in that grapefruit leaves were available from two trees (planted March 23, 1928) growing in very sandy soil near Indio. One of the trees (sample 2) stood between the first and second palm in a row of three Deglet Noor palms and although not actually included in any one of the three irrigation basins about the palms, the grapefruit tree was so close as to have its drip and no doubt its roots included in two of the basins. Each basin received 125 pounds of copper sulfate on November 20, 1930. The soil treatment severely injured the palms although they made an appreciable recovery. The grapefruit tree, as recently as February, 1934, showed no injurious effects. The second of the grapefruit trees grew in soil between two palms, the irrigation basins of which each received 75 pounds of copper sulfate on December 15, 1931. The leaf sample (sample 4) was collected on May 16, 1933, at which time some of the leaves were falling prematurely, indicating that some degree of injury had taken place.

[6] Samples of soil were obtained by S. C. Dorman, of the Citrus Experiment Station, and D. A. Newcomb, of the Corona Foothill Lemon Company, near the trees from which samples 4 and 5 were collected.

TABLE 3

COPPER CONTENT OF LEMON LEAVES COLLECTED FROM VARIOUS GROVES IN SOUTHERN CALIFORNIA

Sample No.	Place and date of collection	Treatment	Cu in dry matter*
			p.p.m.
1	East Whittier, Jan. 7, 1932	Mature; no HCN used	5.6
2	San Dimas, Jan. 8, 1932	Mature; no HCN used	6.7
3	Olive, Jan. 19, 1932	Mature; no HCN used	4.3
4	Anaheim, Jan. 19, 1932	Mature; no HCN used	9.4
5	La Habra, Jan. 19, 1932	Mature; no HCN used	10.0
6	Yorba Linda, Jan. 19, 1932	Mature; no HCN used	6.5
7	Riverside, Jan. 6, 1932	Immature; no HCN used	2.2
8	Riverside, Nov. 2, 1932	Mature; control for 9 and 10; no HCN used	3.8
9	Riverside, Nov. 15, 1932	Mature; very heavy leaf drop after HCN fumigation	7.6
10	Riverside, Nov. 2, 1932	Mature; 5 pounds copper sulfate in basin about tree. Sample consists of the uninjured leaves. Tree injured by HCN fumigation	11.1

* Samples of dry matter ranged from 22 to 42 grams, with an average of about 30 grams.

It is remarkable that the accumulation of copper was not much greater than in the control trees. The use of abundant water in the large, deep basins, together with the deep, sandy soil and the dilution factor of vigorous tree growth probably saved the trees from severe copper injury. In each case the total iron content of the dry matter was reduced when the copper content was increased. However, this does not necessarily indicate that one ion had any specific effect on the absorption of the other. The difference in the phosphorus in samples 3 and 4, without further experiments, cannot be considered significant.

The data given in table 3 were secured from analyses of samples of lemon leaves obtained from various locations. The data serve to show the relatively small concentrations of copper in lemon leaves, especially at Riverside. The data for samples 8, 9, and 10 indicate how injury resulting from fumigation in a given grove was associated with higher contents of copper. The very heavy leaf drop (sample 9) no doubt destroyed leaves with a higher copper content than those remaining on the tree after the HCN injury took place, so that the data for our sample represents the copper content of leaves at or below the threshold of injury. It is difficult to say whether or not the trees from which samples 1 to 6 were taken would have suffered from HCN fumigation because of differences in the environmental factors.

The data in table 4 (samples 1 and 2) are of interest in comparing the copper content of leaves from exanthema-affected orange trees and from adjacent healthy trees. The leaves from control trees contained nearly 15 parts per million of copper, while the leaves from exanthema-affected trees contained only about 7 parts per million. Throughout these studies, one gains the impression that exanthema on different soils and in different climatic conditions may occur when the copper content of the tree is at one level in one location and at another in another location, so that it becomes important to secure control samples as close as possible to the diseased area.

The leaves in sample 4 contained more copper than the control leaves, although most of the injured leaves had already fallen to the ground and were not included. In no case were leaves picked up from the soil because of the danger of copper contamination and of losses in the dry matter of the leaves. As a measure of precaution against splashing of soil up on to the leaves or the whipping of leaves against the soil, the leaves were picked from well up on the tree. In sample 4 had the samples been picked earlier, there is no doubt but that the copper content would have been much higher. In fact, in sample 4, the leaves were only slightly burned and may be looked upon as being just below the threshold at which severe injury took place.

TABLE 4

COPPER, IRON, AND ASH PHOSPHORUS IN MATURE NAVEL AND VALENCIA ORANGE LEAVES

Sample No.	Place and date of collection	Variety	Treatment	In dry matter*		
				Cu	Fe	PO_4
				p.p.m.	*p.p.m.*	*p.p.m.*
1	Moreno, Oct. 25, 1928	Washington Navel	Control from healthy trees adjacent to exanthema area	14.94	......	......
2	Moreno, Feb. 17, 1928	Washington Navel	Leaves apparently healthy, but from exanthema-affected trees	6.78	......	......
3	Citrus Exp. Sta. plot, May 26, 1933	Washington Navel	Soil received calcium nitrate and covercrop (control); no HCN	9.50	214	1,750
4	Citrus Exp. Sta. plot, May 26, 1933	Washington Navel	Soil basins received 4 to 5 pounds copper sulfate in 1929 to cure exanthema, which it did; trees badly injured by fumigation in autumn, 1932	11.10	180	1,860
5	Riverside, June 23, 1933	Valencia	Trees easily injured by HCN fumigation; no copper treatment; no exanthema at any time	12.80	204	1,810
6	Arlington, grove D, June 1, 1933	Washington Navel	Control, several rows of trees away from treated trees	8.14	......	......
7	Arlington, grove D, June 1, 1933	Washington Navel	Control, several rows of trees away from treated trees, but in a direction opposite to sample 6	10.10	......	......
8	Arlington, grove D, June 1, 1933	Washington Navel	8 pounds copper sulfate per tree-square; applied in furrows before irrigation in 1930; no exanthema at any time	10.75	......	......
9	Arlington, grove D, June 1, 1933	Valencia	8 pounds copper sulfate per tree-square; applied in furrows before irrigation in 1930; no exanthema at any time	21.40	......	......
10	Capistrano, grove E, March 13, 1930	Valencia	Control; exanthema-affected trees; no copper treatment	3.93	......	......
11	Capistrano, grove E, March 13, 1930	Valencia	Exanthema-affected trees that became healthy through the use of 5 pounds copper sulfate applied in each of the four squares about a tree	7.86	158	......
12	Capistrano, grove F, March 13, 1930	Valencia	Control; exanthema-affected trees; no copper treatment	9.93	......	......
13	Capistrano, grove F, March 13, 1930	Valencia	Exanthema-affected trees that became healthy through the use of copper sulfate applied to the soil	21.20	......	......

* Samples of dry matter ranged from 23 to 75 grams, with an average of about 50 grams.

In a certain grove in Riverside, fumigation injury has been the rule for a long time with no apparent reason. Leaves (sample 5) secured from these trees showed nearly 13 parts per million of copper. Bordeaux paste and other copper preparations are frequently used in the treatment of the trunk bark diseases or for protection against their occurrence. It is possible that such applications serve to increase the supply of copper in the leaves and other portions of the tree, and later this may serve as a cause of increased fumigation injury.

It is known that copper is a very effective agent in the precipitation of proteins, and in the light of Guba and Holland's[15] conclusion that HCN fumigation increases the solubility of copper, it is also possible that the injury resulting from fumigation of tissues rich in copper may be due to the increased precipitation of proteins in the cells. Such protein precipitation together with the action of HCN on tissue oxidation may determine whether tissue injury shall be reversible or permanent in nature.

While copper in contact with leaves may, according to Guba and Holland, become more soluble by HCN fumigation, it would appear that it is largely if not entirely the copper within the cells rather than that between the cells or on the leaf surface that determines injury. It should be mentioned in this connection that the extent of the saturation deficit of the leaf tissues may be of importance in the penetration of soluble salts into the leaves, together with solvent agencies such as composition and other properties of tissue fluids.

The possibility of an increased absorption of copper by citrus leaf tissues is seen in the use of copper sulfate in irrigation canals in order to reduce the growth of algae and thereby increase the rate of flow. In certain cases, iron sulfate was found to be effective at first, but the plants soon developed a tolerance so that copper sulfate was substituted for iron sulfate. The lumps of the sulfates were placed in gunny sacks and crushed; hence, when the sacks were immersed in the irrigation water, the finely divided material quickly dissolved and passed out in the water in such excessive amounts as to kill portions of alfalfa in a field. The effect of such water treatments on the copper content of citrus trees may be far reaching, even when the copper sulfate is carefully dripped as a solution into irrigation water in order to increase the flow or to make such water suitable for domestic use. Plants in water canals also may develop a tolerance to copper so that larger and larger amounts may be necessary as the use of copper is continued.

As shown in table 4, samples 6 to 9 inclusive were obtained from trees that were declining, but were unaffected with exanthema. Since none of the trees could be said to be in the best state of health, some were given

TABLE 5

COPPER CONTENT OF HEALTHY AND DISEASED LEMON FRUITS*

Sample No.	Place and date of collection	Fruit samples	Cu in dry matter†
			p.p.m.
1	Whittier, Jan. 8, 1932	Peel only, full-size, ripe fruit	3 40
2	Whittier, Jan. 12, 1932	Peel only, full-size, ripe fruit	3.70
3	Whittier, Jan. 12, 1932	Pulp and seed only, full-size, ripe fruit	4.70
4	Riverside, Nov. 3, 1932	Control; peel, pulp, and seed; full-size, ripe fruit	2.80
5	Riverside, Nov. 3, 1932	Control; fruit not full-size, green, 4 cm diam. × 6 cm long; peel, pulp, and seed	3 00
6	Riverside, Nov. 3, 1932	Same as sample 5, except that the soil about tree received 5 pounds copper sulfate	3.80
7	Riverside, Nov. 3, 1932	Fruit from another tree like the one from which sample 6 was collected	3.90
8	Riverside, Nov. 3, 1932	Ripe fruit; peel, pulp, and seed; 5 pounds copper sulfate applied in basin about tree	3.30
9	Riverside	Control; ripe fruit	3.74
10	Citrus Exp. Sta. and Rubidoux plots	Control; ripe fruit (Eureka)	3.58
11	Citrus Exp. Sta. and Rubidoux plots	Control; ripe fruit (Eureka on sour)	4.90
12	Carpinteria, June, 1927	Control; ripe fruit from healthy block of trees near diseased trees	4.34
13	Carpinteria, grove A	Fruit from exanthema-affected trees; ripe fruit	1.66
14	Carpinteria, grove B, Jan. 29, 1926	Fruit from exanthema-affected trees; ripe fruit	3 20
15	Carpinteria, grove C, June, 1927	Fruit from exanthema-affected trees; ripe fruit	2.08
16	Carpinteria, adjoining grove B, June, 1927	Good fruit from healthy trees among exanthema-affected trees; ripe fruit	2.95
17	Goleta, Nov. 16, 1927	Ripe fruit; very thick peel; pulp dry; affected with exanthema	3.75
18	Goleta, Dec. 1, 1927	Fruit immature; all sizes mixed together; very thick peel; pulp dry; affected with exanthema	1.91

* Seed-free basis, unless otherwise stated.

† Samples of dry matter ranged from 40 to 120 grams, with an average of about 80 grams.

soil applications of copper sulfate to learn if it would bring about improvement. Very little increased copper content occurred in sample 8, but in sample 9 the increase was about equal to the original content.

The leaves (sample 10) from one grove of exanthema-affected trees on very heavy, black soil at Capistrano showed about 4 parts per million of copper, while those (sample 11) from trees in the same area that

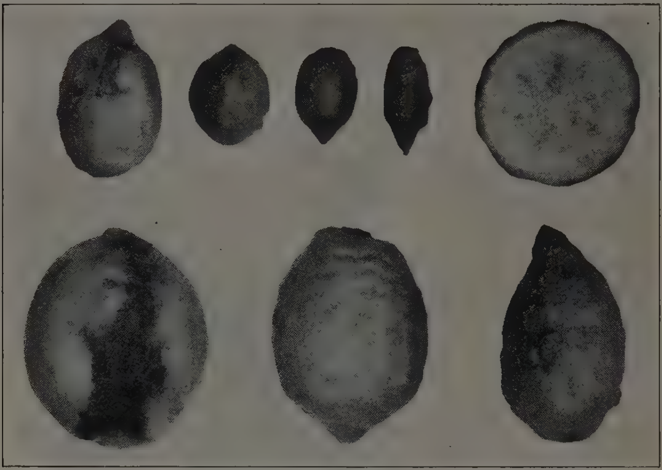

Fig. 1.—Lemon fruits (sample 18, table 5) affected with exanthema. The fruits were irregular in shape and topography. The rind was scaly from cracked, gum-soaked areas, and was very thick, while the pulp was dry and worthless.

were made healthy by the application of copper sulfate to the soil, contained nearly double this amount of copper.

In another grove at Capistrano the leaves (sample 12) from exanthema-affected trees contained nearly 10 parts per million of copper, while those (sample 13) from diseased trees that were made healthy by the application of copper sulfate to the soil, contained about double this amount. Samples 10 to 13 illustrate the variation in the copper content of leaves from diseased trees in groves a few miles distant from each other. Table 4 (samples 3 and 4) again shows the reduced iron and increased phosphate in cases where copper has brought the trees back to health.

Table 5 gives the copper content of healthy and diseased lemon fruit. In each case at least 25 lemons were used in preparing the fresh samples from the dry matter of which an aliquot was taken for the copper determination. Table 5 indicates the relatively small concentration of

TABLE 6

INORGANIC COMPOSITION OF HEALTHY AND EXANTHEMA-AFFECTED WHOLE LEMON FRUITS

Sample No.	Fruit samples, place, and date of collection	Ash as a per cent of dry matter	Ash constituents as a per cent of total ash						
			Na	K	Ca	Mg	Cl	SO_4	PO_4
1	Exanthema-affected, ripe lemons from Carpinteria, collected in grove A, Jan. 29, 1926	3.81	7.12	31.94	11.11	3.62	1.95	3.97	14.66
2	Same as sample 1, but collected in grove B	3.64	10.05	32.91	10.73	2 91	1.30	3 59	16.93
3	Same as sample 1, but collected in grove C. A composite of ripe and immature fruit	3.58	11.57	30.05	11.52	3.07	2.09	3.28	18.92
4	Ripe lemons from healthy trees, Sespe, Jan. 28, 1926	4.38	7.15	30.19	15.27	3.01	0.31	3 55	11 50
5	Ripe lemons from healthy trees, upper rootstock plots, Rubidoux Station, Jan. 30, 1926	4.81	9.38	30.32	13.90	2.59	0.78	3.13	12.43
6	Ripe lemons from healthy trees on fairly saline soil, Irvine, Sept. 18, 1925	4.88	7.79	29.95	13.08	2 66	7 01	3.33	13.81

copper in lemon fruit. The application of copper sulfate to the soil (samples 6 to 8) is reflected but slightly in the copper content of the fruit.

The importance of copper for fruit affected with exanthema is seen in samples 12 to 18 inclusive. No control other than sample 12 was available for samples 17 and 18. Figure 1 illustrates the condition of lemons (sample 18) when affected with exanthema. The fruit is abnormally shaped and the peel is thick and irregular in topography.

TABLE 7

Copper Content of Healthy Washington Navel and Valencia Oranges

Sample No.	Place and date of collection	Fruit samples	Cu in dry matter*
			p.p.m.
1	Highland, Feb. 24, 1928	Navel; whole fruit; trees in poor condition	2.77
2	Moreno, Oct. 25, 1927	Navel; whole fruit; trees in good condition	4.36
3	Highland, May 3, 1927	Valencia; peel and pulp; trees in poor condition	1.78
4	Highland, May 3, 1927	Valencia; peel and pulp; Tahiti sweet orange root, lemon trunk	2.22
5	Riverside, plot F, Rubidoux, Jan. 31, 1927	Valencia; peel and pulp	3.28
6	Riverside, plots N and F, Rubidoux, Dec. 27, 1926	Valencia on sour; peel and pulp	2.56
7	Riverside, Rubidoux, Dec. 27, 1926	Valencia on sweet; peel and pulp; upper rootstock	3.02
8	Riverside, Rubidoux, Dec. 27, 1926	Valencia on Trifoliate orange; peel and pulp; upper rootstock	2.27

* Samples of dry matter range from 60 to 112 grams, with an average of about 105 grams.

Table 5 suggests, although it does not show conclusively, that there may be a range for a given location of diseased trees below which the copper content of the fruit may not fall without showing symptoms of the disease. In addition, it was found that the total iron content of the dry matter of sample 9 was 35 parts per million and of sample 10, 26 parts per million.

It may be of interest to consider the results (table 6) of analyses of healthy and exanthema-affected lemon fruits. One outstanding difference lies in the ash content of the dry matter, which is much greater in healthy fruit. It has been found that the ash of diseased fruit contains slightly less calcium, but more phosphate than that of healthy fruit. On a dry weight basis the PO_4 content of the two groups of samples, namely, diseased and healthy, varies within the same range. A further comparison on a dry-weight basis shows a larger percentage of potassium and more calcium in the fruits from the healthy trees as compared with the fruits from diseased trees.

The results of preliminary copper determinations of healthy Washington Navel and Valencia oranges are given in table 7. The lowest copper content was 1.78 parts per million in sample 3 from Highland, and the highest was 4.36 parts per million in sample 2 from Moreno. The iron content of sample 1 was 68 parts per million. The data emphasize the need of a greater degree of subdivision of the fruit because of the small concentrations of copper involved. Division of the fruit into

TABLE 8

COPPER CONTENT OF PEEL AND PULP OF HEALTHY VALENCIA ORANGES*

Samples, place, and date of collection	Portion of fruit	Cu in dry matter†
		p.p.m.
Sample 1, Orange County, Aug. 5, 1931 (79 fruits)	Calyx quarter of peel	3.97
	Second quarter of peel from calyx end	3.82
	Third quarter of peel from calyx end	4.57
	Stylar quarter of peel	4.54
	Calyx half of pulp	3.66
	Stylar half of pulp	4.31
Sample 2, Pathology plot, Citrus Experiment Station, Aug. 5, 1931 (100 fruits)	Calyx quarter of peel	2.38
	Second quarter of peel from calyx end	3.59
	Third quarter of peel from calyx end	2.83
	Stylar quarter of peel	4.75
	Calyx half of pulp	3.94
	Stylar half of pulp	3.53

* Seed-free basis.

† Samples of dry matter averaged about 100 grams each.

peel and pulp was therefore made in subsequent determinations, and in some cases these were further subdivided, as shown in table 8. This method of dividing the fruit is an outgrowth resulting from the experiments of Haas and Klotz[19] on gradients in the composition of citrus fruit.

Table 8 gives the results of copper determinations in various portions of healthy and diseased Valencia oranges. Samples 1 and 2 represent various aliquots of the dry matter of 79 and 100 Valencia oranges obtained from Orange and Riverside counties, respectively. Haas and Klotz[19] have tabulated the organic and inorganic contents, not including copper, of the portions mentioned in table 8. The results for copper in the present paper suggest a greater concentration of copper in the stylar than in the calyx portion of the peel in both samples.

Twenty-five fruits were used in preparing each of the remaining dried samples, data for which are given in table 9. Samples 1 and 2 were obtained in San Diego County. The data for these samples show that the exanthema-affected peel and pulp both contain smaller concentrations of copper than the corresponding portions of fruit from healthy trees.

TABLE 9

Copper Content of Various Portions of Healthy and Diseased Valencia and Navel Oranges*

Sample No.	Place and date of collection	Variety	Condition and treatment of trees and soil	Cu in dry matter†	
				Peel	Pulp
				p.p.m.	*p.p.m.*
1	San Diego County, Jan. 15, 1932	Valencia	Healthy trees; no treatment	6.10	2.73
2	San Diego County, Jan. 15, 1932	Valencia	Trees affected with exanthema; no treatment	2.30	1.56
3	Capistrano, grove A, March 13, 1930	Valencia	Trees affected with exanthema; no treatment	10.04	16.40
4	Capistrano, grove A, March 13, 1930	Valencia	Trees once affected with exanthema; cured by 5 pounds copper sulfate in each tree-square or 20 pounds per tree; trees badly defoliated by fumigation; fruit scorched	12.30	13.10
5	Capistrano, grove A, March 13, 1930	Valencia	Trees as in sample 4, but never fumigated	62.07	30 60
6	Capistrano, grove B, March 13, 1930	Valencia	Healthy trees; no exanthema within several miles; no treatment	8.66	3 00
7	Capistrano, grove B, March 13, 1930	Valencia	Trees affected with exanthema; no treatment	7.29	18.07
8	Capistrano, grove C, March 13, 1930	Valencia	Trees affected with exanthema; no treatment	16.58	10.72
9	Capistrano, grove C, March 13, 1930	Valencia	Trees affected with exanthema; not cured as yet by 5 pounds copper sulfate in each tree-square	12.60	5.07
10	Moreno, grove A, Nov. 7, 1930	Navel	Trees badly affected with exanthema; nearly entire crop lost by fruit splitting; no spray or soil treatment	1.64	1.18
11	Moreno, grove A, Nov. 7, 1930	Navel	Trees once badly affected with exanthema; sprayed with bordeaux mixture in 1928; fruit in 1930 apparently free of disease	2.04	1.28

* Seed-free basis.

† Samples of dry matter ranged from about 30 to 130 grams, with the majority over 100 grams. Samples of healthy and diseased fruits were approximately equal in size.

Samples 3, 4, and 5 were obtained on a different type of soil at Capistrano. Fruit (sample 3) from exanthema-affected trees that received no copper treatment contained less copper in the peel than the fruit from trees on copper-treated soil (samples 4 and 5). The peel of sample 4 showed only slightly more copper than sample 3. This may be due to the defoliation of the trees by the fumigation, with reduced absorption as a result. Since the fruit was severely burned when fumigated, this may have checked the absorption of copper as compared with the fruit of sample 5, which was not fumigated and hence was not burned. Many of the most severely injured fruits had fallen prior to the collection of the fruit sample. It is possible that the fruits remaining on the trees contained less copper than those that had fallen. Experience has shown the peel to give more reliable results than the pulp of Valencia oranges from which the seeds have been removed on account of the variability in their number. The copper content of sample 7 shows a slightly reduced copper content in the peel of exanthema-affected fruit as compared with sample 6.

Samples 8 and 9 were from badly diseased trees. Even though copper sulfate was used in the case of sample 9, the appearance of the trees and fruit, and the copper content of the peel, indicate that either sufficient time had not elapsed since the application of the copper sulfate to permit recovery or that recovery was not possible. Copper sulfate applied to soil may not benefit all exanthema-affected trees, owing to the lack of an adequate root system to enable recovery to take place.

Sample 11 shows only the slightest increase in copper content, even though the trees and fruit apparently were cured of all exanthema symptoms. These values for sample 11, as well as the other data in table 9, show the great variation that exists in the copper content of fruit peel and pulp obtained from various locations and the advantages of separating the peel and pulp. The data further indicate the necessity of conservatism in drawing conclusions from such studies.

Additional data were secured regarding the soil bearing the trees, from which samples 10 and 11 were obtained. The pH of the first 6 inches of soil was 7.03, while that of the third foot was 7.30. In some locations where the exanthema was severe, it was possible to push a soil tube 6 feet into the soil by hand. Nitrate determinations of 1 to 5 water extracts of the dry soil showed 16 parts per million NO_3 in the first foot, 21 parts per million in the second, 12 parts per million in the third, and 13 parts per million in the fourth. While these values for NO_3 show an ample supply of nitrate, they do not show an excessive supply. This is in harmony with the conclusions of other investigators, namely, that nitrate of itself is not the cause of exanthema or mottle-leaf.

The question frequently is asked, "Is it the copper remaining on the soil or the increased copper within the tree that brings about fumigation injury?" When copper sulfate is added to the soil, it may require several years before it has changed sufficiently so as to be unrecognizable to the

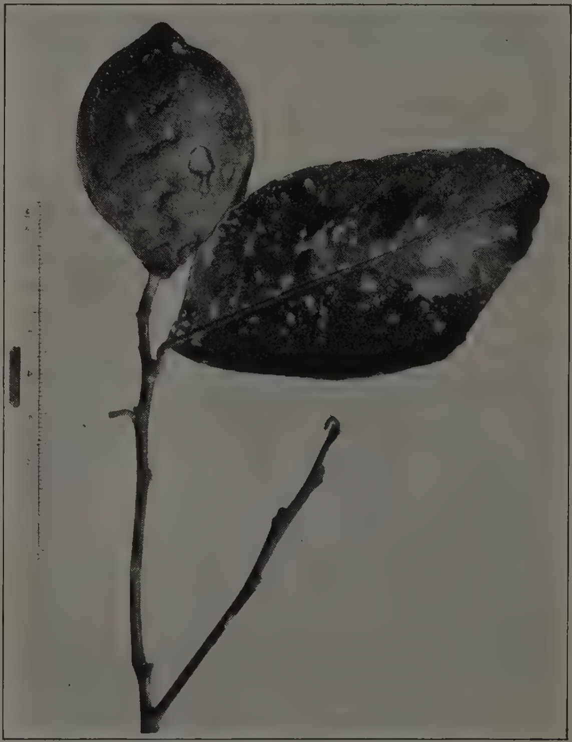

Fig. 2.—Lemon leaf and fruit (from tree of which the skirt was treated with bordeaux mixture for brown rot control) showing injury from the fumigation of a nearby lemon grove.

eye. (The finely pulverized form disappears more rapidly than the lumpy form.) After 3 to 5 years following the application of 5 pounds of copper sulfate to the soil, there should be very little, if any, soluble copper on the soil surface. The fact that fumigation injury frequently occurs after such a prolonged interval indicates the importance of the internal copper in the tree. Furthermore, data have been presented which suggest that some trees that are injured year after year by fumi-

gation may contain an increased content of copper in the leaves without copper having been intentionally applied.

Another aspect of the use of bordeaux mixture on the skirts (lower portion of crown) in the control of brown rot came to the attention of the authors in February, 1934. A lemon grove was fumigated without injury on the evening of the same day on which the skirts of a grove of lemon and orange trees on lower adjoining land were sprayed with

Fig. 3.—Lower part (skirt) of Navel orange tree injured when treatment with bordeaux mixture was followed within 24 hours by HCN fumigation in a nearby grove. Note the large number of dead leaves on the ground.

bordeaux mixture. Serious injury resulted to the latter. The fumigated grove was not only situated on higher land than the sprayed grove but was separated from it by a wide highway. The lower rows of sprayed trees were 8 in number and were planted at right angles to the highway. The sprayed trees were double-planted, there being about 45 lemon trees in the upper portion of the sprayed rows and about 28 Navel orange trees in the lower portion. The skirts of adjoining Navel orange trees were also sprayed with bordeaux mixture but subsequent to the fumigation of the lemon grove previously mentioned, and showed no injury.

Figure 2 shows the typical fumigation injury of the leaves and fruit of the skirts of the lemon trees sprayed with bordeaux mixture but to which the HCN from the fumigation of the lemon trees above the road may have drifted. Figure 3 gives some idea of the leaf fall following the injury to the Washington Navel orange trees also sprayed with bordeaux mixture. The effect of leaf abscission of the orange tree skirts in making the trees barren underneath when normally the crown of the trees

extended to the ground, is seen beneath the white line in figure 4. As the lemon grove above the road was fumigated, the gas may have drifted down the slope and injured the skirts of all of the trees in the rows. These rows consisted of 45 lemon trees at the upper end and 28 Washington Navel orange trees at the lower end. The degree of injury did not become smaller with increasing distance from the road but on the contrary was equally severe at any place in the rows of 73 trees each. Adjacent unsprayed Washington Navel orange trees facing the three sides of these 8 rows were not injured. The possibility exists that the injury of the trees may have been brought about because of the nature of the bordeaux mixture used without the action of an HCN drift, but this is unlikely in that the injury appeared to be typical of HCN and because trees sprayed after the fumigation were unaffected. However, the possibility is not excluded that the two lots of bordeaux mixture may have been different in composition.

Fig. 4.—Navel orange trees severely injured when the lower part was sprayed with bordeaux mixture for brown rot control followed within a day by HCN from a fumigated lemon grove a considerable distance away on higher ground. Below the white line is seen the extent of defoliation of the skirts of the trees.

If further cases are found to confirm this one as being due to the drift of HCN downhill with its injurious action upon foliage sprayed with bordeaux mixture, it would become of great importance to growers on sloping areas to know at all times precisely what their neighbors intend doing in order to avoid such HCN damage.

The owner of the grove from which samples 10 and 11 (table 9) were secured finds that in his various groves he may fumigate very soon (within 6 months) after using bordeaux mixture on the trees. Possibly this is due to the fact, as the data suggest, that his trees are somewhat deficient in copper.

It may be of interest here to compare the iron and copper content of citrus with that of other leaves and fruits. A comparison of the ratio of iron to copper in winter pears with that in other classes of foodstuffs was made by Moore,[27] who found that the ratio for pears ranged from 0.523 :1 to 0.755 :1, while the ratios for four different classes of foodstuffs investigated by Peterson and Elvehjem[31] and Lindow, Elvehjem, and Peterson[23] as given by Moore[27] ranged from 3.53 :1 to 57.5 :1.

In table 1 the ratio for sample 1 (orange leaves) was about 50:1, that for sample 2 (lemon leaves), 21:1, and for sample 3 (lemon leaves), about 6:1. The ratios for grapefruit leaves in table 2 were as follows: Sample 1, 26:1; sample 2, 15:1; sample 3, 27:1; and sample 4, 16:1.

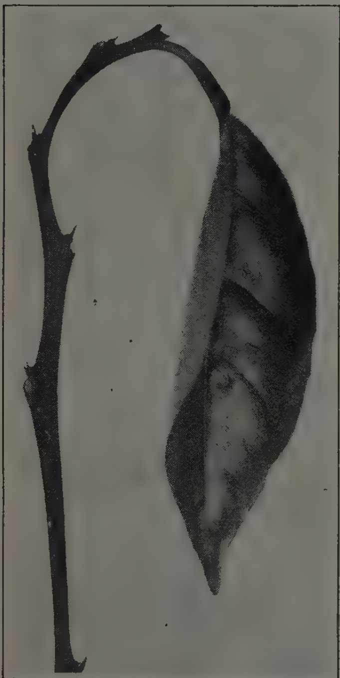

Fig. 5.—S-shaped shoots on Valencia orange trees affected with exanthema in sand cultures.

Fig. 6.—Gum exúdation from Valencia orange twigs affected with exanthema. Note the killing of the terminal portion of the twig. The trees were grown in sand cultures.

Samples 2 and 4 were from trees growing on soil to which copper sulfate was applied, while samples 1 and 3 were from control trees. The copper nearly halved the ratio. In control Washington Navel orange leaves (table 4, sample 3), the ratio was 22:1, while sample 3 from trees on copper-treated soil was only 16:1.

We have gained considerable chemical background with regard to the occurrence of copper in citrus, the relation of copper to exanthema in the field, and of injury by fumigation when copper is present in slight excess. We shall now consider a copper deficiency (under controlled conditions) as a cause of exanthema.

On May 21, 1920, Valencia orange trees grafted on sour-orange stocks were planted, bare root, in 12 large tanks of pure silica sand. The tanks were 3 feet 8 inches in diameter by 4 feet deep. Below this the bottom

Fig. 7.—Exudation of gum from Valencia orange twigs affected with exanthema while growing in sand cultures. The unbroken gum pockets are marked in the photograph.

tapered 6 inches to a perforated plate beneath which was a 4-inch elbow that connected with a 2-inch galvanized iron pipe. Each tank had its individual drainage outlet in a trench. Crushed quartz rock was placed in the bottom of each tank as a drainage system for the sand. No organic matter was used except the small amounts of iron tartrate.

The sand received a nutrient solution (Haas[16]) including A-Z, which contained no added copper. The culture solution was mixed in large, paraffin-coated wooden barrels and generously supplied every few days to each of the tanks.

During the early growth in sand cultures no unusual symptoms appeared, owing perhaps to the large carryover of inorganic constituents from having grown for two years in soil. Until 1926 the trees were growing vigorously, and the indications were such that all we could conclude from this experiment was that citrus trees could be grown for many years in sand cultures without organic matter, even during the heavy production of fruit. However, in 1926 and succeeding years the large size of the trees permitted deficiencies to make themselves apparent.

In 1927 pronounced symptoms of exanthema appeared on several of the trees. Figure 5 shows the distorted or S-shaped appearance of many vigorously growing shoots. Many shoots began dying back from the terminus and exuding gum, as shown in figure 6. Gum pockets or blisters were evident on succulent shoots, as shown in figure 7. As the disease became more severe, a resinous excrescence (fig. 8) stained the affected young shoots, many of which died back, producing a witches' broom effect. Even the leaves were finally covered on the ventral side with the resinous stain (fig. 9). On some trees, many of the resin-stained leaves were becoming chlorotic, a result which is in harmony with the findings of Anderssen[2] on deciduous trees suffering from copper deficiency in the field. Measurement of 100 leaves from healthy and diseased trees showed the average leaf of a healthy tree as being 3.9 cm wide and 7.8 cm long, and that of a diseased tree as being 3.7 cm wide and 9.7 cm long. The leaves tend to become narrower in proportion to the length.

The pH of the drainage water from the tanks ranged from 6.6 to 7.6, the nitrate from 351 to 1,226 parts per million, and the total solids 651 to 1,600 parts per million. The addition of 0.05 part per million of copper as sulfate to the culture solution gradually overcame the disease on all except one tree which, while not cured, was distinctly improved. While the use of copper sulfate was being tried, the studies were abruptly abandoned on July 1, 1928, because of the necessity of at once moving from the Rubidoux laboratory to the Citrus Experiment Station. This prevented further studies in getting at the facts surrounding this problem of exanthema and its relation to copper. Such tank experiments are expensive, of necessarily long duration, and require tedious and constant attention, because any one of many factors in a day may undo the work of years.

These tank experiments, together with field and laboratory work,

Fig. 8.—Resinous excrescence on young Valencia orange twigs from trees affected with exanthema in sand cultures.

Fig. 9.—Resinous excrescence, particularly on the ventral surface of Valencia orange leaves affected with exanthema while the trees were grown in sand cultures.

have shown minute amounts of copper as being essential for healthy growth in citrus, and that exanthema may be brought about by a deficiency in the supply or availability of the copper in the tree. No experiments have as yet been carried on in regard to the solubility of copper in citrus trees or in the soils in which the trees are grown. The results thus far indicate that the beneficial effects of copper sulfate came about by the correction of a deficiency of copper as such rather than by the precipitation of toxins or the killing of microörganisms. The amount of copper in a citrus tree that will still permit healthy growth seems to vary with other factors such as soil, climatic conditions, etc. Vigorously growing citrus trees in most cases withstand a considerable increase in the copper content of their tissues without injury unless fumigation enters in as another factor to be considered.

SUMMARY

A study was made of the copper content of citrus leaves and fruit by means of a method that permits of the accurate determination of minute amounts of copper.

The amount of copper required for healthy growth varies with the soil and climatic factors, although the solubility of the copper may later be found to be of considerable importance.

Small amounts of copper suffice to maintain healthy growth in citrus, although somewhat larger amounts appear to do no injury. For example, in a very sandy soil a single 125-pound application of copper sulfate to a large basin close to the drip of a grapefruit tree did not produce injury, although an application of 75 pounds to the soil close to another grapefruit tree brought about considerable injury. However, amounts of copper sulfate above 5 pounds per tree-square are to be used only in special cases, as they may cause injury on many soils, according to the nature of the soil and root distribution. For the control of exanthema, spraying with bordeaux mixture makes unnecessary the addition of copper sulfate to the soil.

Leaves of exanthema-affected trees show a reduced copper content. These results with citrus confirm those of Oserkowsky and Thomas[30] on pears. The copper content of citrus fruit was best studied by analysis of the peel and pulp separately after removal of the seeds. Although more work is to be desired, the results thus far obtained for citrus indicate that a deficiency of copper occurs when the fruits are affected with exanthema.

It is very evident that exanthema can be readily controlled in the field by relatively small amounts of copper; but when the analytical data are examined there is considerable overlapping, and the clear,

definite results that might be hoped for are not to be had. The results show rather clearly that considerable variation exists in the copper content of leaves and fruit from different localities and even different groves in a locality. Because of this, it appears that analyses for copper content will give little indication of whether or not a grove might be suffering from a lack of copper and on the verge of having exanthema or whether there may be so much copper present that HCN fumigation would likely result in injury. The importance of carefully chosen control leaves and fruit in work of this kind cannot be overemphasized.

By means of 12 large tank cultures filled with sand, typical symptoms of exanthema were produced: gum pockets and gum exudation appeared on vigorous shoots; a resinous excrescence was found on young twigs which died back and gave rise to short, new shoots that resembled a mild witches' broom effect; leaves were longer and narrower than on healthy trees; the ventral sides of diseased leaves were resin-stained; and the vigorous shoots of diseased trees made an S-shaped growth. During advanced stages of exanthema, many of the leaves became somewhat chlorotic, and their abscission followed.

Copper is essential for health in citrus, and when deficient, symptoms of exanthema make their appearance. When copper is used to correct exanthema, fumigation should not be practiced before sufficient time has elapsed until growth and reproduction, soil precipitation, and leaching have had time to reduce the somewhat too great an increase in the absorption of copper down to a concentration with which HCN will not react sufficiently to bring about injury.

Citrus trees that have year after year shown fumigation damage in comparison with trees in other groves were found to contain increased amounts of copper. However, the studies in this paper upon the relation of copper to fumigation injury should be regarded as preliminary.

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PHYSIOLOGICAL GRADIENTS IN CITRUS FRUITS

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PHYSIOLOGICAL GRADIENTS IN CITRUS FRUITS[1,2]

A. R. C. HAAS[3] AND L. J. KLOTZ[4]

INTRODUCTION

THE VARIOUS PORTIONS OF CITRUS FRUITS, particularly the regions near the calyx and stylar ends, differ not only in their morphology but also in their physical and chemical characters. The active acidity of various organs of plants forms a gradient that has been given considerable attention by Haas,[(11)] Gustafson,[(9, 10)] and Hurd-Karrer.[(17, 18)] The present paper presents evidence of the existence of physical and chemical differences and gradients in citrus and other fruits, and attempts to explain these differences in citrus fruits by a consideration of the structure and function of the parts concerned.

The functions of the vascular system of plants are support, and the conduction and distribution of solutes. Soluble materials that remain in the vascular stream after lateral absorption by the cells adjacent to the tracheal tubes, concentrate in the tissues near the terminals of the vascular strands. Cells in this region necessarily receive the surplus of the solutes. The location of these terminals is not always easily recognized because of branching and anastomosing, but the concentrations of ions as revealed by chemical analyses may indicate regions in which numerous vascular strands terminate. Ross[(26)] has described the analogy of the morphology of the fruit carpels and that of the citrus leaf. The main lateral veins of the leaf join at the margin and proceed to the leaf tip, which region, as shown by Haas,[(13)] becomes the principal place of deposition for soluble materials. It will be shown that similar regions are found in the citrus fruit, the structure of which is composed of a number of modified leaves coalesced to form the whole. Mature leaves on twigs will be considered only as they may affect the gradients in the fruits.

Susceptibility to abscission decreases as fruit matures, injuries due to malnutrition manifesting themselves by other symptoms. Localization of these injuries in certain parts of the fruit suggested a study of the physical and chemical composition of the susceptible and resistant parts.

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Analyses showed the existence of relatively large differences. Furthermore, lesions caused by certain fungi, injuries due to fumigation and oil spray, and physiological breakdowns, such as granulation and storage spots, are more commonly found on and in the calyx end of the fruit than elsewhere, while certain other fungus spots and endoxerosis occur more commonly in the stylar end. One of the principal objects of this investigation was to find whether there are, in the fruits, differences in the physical or chemical composition which might serve as a basis for explaining the characteristic localization of the lesions above mentioned.

While there is considerable information available concerning the composition and nutrition of leaves and twigs, there has been little correlation of this information with the nutrition of the fruit.

Analyses of citrus fruit by previous workers have been concerned largely with total acids, oil content, proteins, reducing and total sugars, total ash of the whole fruit, and the sugars and acid to total solids ratio of the juice. These are discussed in the publications of Chace, Tolman, and Munson,[4] Chace, Wilson, and Church,[5] Wilson and Young,[29] Chace and Church,[2, 3] Poore,[24] and Collison.[6]

METHODS OF ANALYSIS

Fruit juices were extracted in some instances by means of a hand extractor and in others with a motor-driven extractor, only one method, however, being employed in a set of comparative determinations. The extracts were centrifuged at high speed for 15 minutes to free them from suspended material. Any oil that collected at the top was discarded and the supernatant liquid decanted into clean, dry glassware.

Density of the fruit juice was determined by weighing in an adjusted Gay-Lussac specific gravity bottle of approximately 25-cc capacity, and relating this weight to the weight of an equal volume of distilled water.

The capacity of juices to fix iodine was determined by adding 25 cc of an iodate-iodide solution (60 grams KI and 5.4 grams KIO_3 per liter) and 20 cc 5N H_2SO_4, to 100-cc samples, shaking, and allowing them to stand 10 minutes. At the end of that period the free iodine remaining was titrated against N/10 sodium thiosulfate solution, using starch as an indicator. The result was subtracted from a blank determination in which 100 cc of distilled water was used instead of fruit juices. This value multiplied by the iodine equivalent of the thiosulfate solution represented the iodine fixed. The procedure is taken from a portion of the method for reducing sugars by Shaffer and Hartmann.[27]

All fruit samples used for analysis were debuttoned and wiped free of dust. The analytical methods employed in the determination of inorganic constituents were those used by Haas[13] in previous work. The

results were calculated on a dry-weight and ash basis and in some cases on a fresh-weight basis. Some of these computations have been omitted in order to conserve space in the tables, although they may be readily recalculated from the given data.

All peel and pulp samples of fruit were dried at 80° C without the loss of juice from the pulp. This was done by separating the fruit at considerable intervals of space on clean, heavy paper in well-ventilated ovens. When sufficiently dry the pulp mass of a single fruit was laid between layers of heavy paper, broken open, and further dried. Unless otherwise stated, the seeds were removed from the pulp prior to grinding the sample.

To obtain the sap of the peel, the peel was carefully removed, placed in paraffined bags, frozen 18 hours at a temperature of 20° F, thawed, subjected to 25,000 pounds pressure per square inch in a hydraulic press, and centrifuged.

The osmotic values of the juices were obtained from the freezing-point depression corrected for the degrees of under-cooling, according to the method of Harris and Gortner.[16]

The relative oil content of the peel of the calyx and stylar ends of fruit was determined by a modified Soxhlet extraction of equal surfaces of peel. Equal numbers of disks were cut from the two ends, weighed, and placed into extraction thimbles with other materials in the following order: a thin layer of pure asbestos fibers, a layer of a mixture of pure silica sand and anhydrous $CuSO_4$ sufficient to absorb the water of the sample, another layer of asbestos, the sample disks of fruit peel, and another layer of asbestos on the top. The prepared sample was extracted for 48 hours with anhydrous ether in a weighed flask. The disks were removed, ground with quartz sand in a mortar, replaced in the extraction thimble with the aid of anhydrous ether, and extracted for an additional period of 24 hours. The condenser thimble and thimble holder were removed, and the ether was allowed to evaporate at a low temperature. The flask and oil were then weighed.

The method used in the determination of the relative permeability of fruits was that adapted by Klotz.[21]

The rate of evaporation or water loss from the stylar and calyx halves of fruit was measured by direct weighing. In one procedure the fruit was cut along the equatorial line and the cut surfaces were dipped in powdered boric acid to retard the growth of microörganisms. The fruit was then placed with cut surfaces downward on glass plates covered with paraffin paper. In a second method the evaporation was excluded from one end or the other by dipping in paraffin of low melting point, as was done by Markley and Sando[23] with apples. The fruits, with un-

paraffined ends upward, were set in small cardboard cylinders on glass plates, and groups of a dozen or more fruits weighed together. It may be mentioned here that the equatorial line was determined by measuring with a flexible celluloid ruler half the surface distance from the center of the cut stem to the center of the stylar scar. It was found by actual volume measurements, using a water displacement method, that the halves thus determined were remarkably similar in volume.

GRADIENTS IN COMPOSITION OF CITRUS PULP JUICE

H-ion Concentration, Specific Gravity, Carotinoids, and Iodine Fixation.—There were no consistent differences in the pH or the buffer action of the juice of calyx and stylar halves of mature Valencia orange pulp. In three lots of 35 oranges each, the juice of both halves gave the values: 3.85, 3.90, and 4.20, respectively. In another similar lot of 35 oranges the pH of the juice of the calyx halves was 4.15 as compared with 3.80 for that of the stylar halves. The juice of both halves of lemons in the silver stage showed pH values of 2.55. These results for lemons are in agreement with those of Bartholomew.[1]

TABLE 1

SPECIFIC GRAVITY OF LEMON, GRAPEFRUIT, AND ORANGE JUICES

Type of fruit	Calyx half	Stylar half
	sp. gr.	*sp. gr.*
Early silver-stage lemons	1.0347	1.0343
Immature Marsh grapefruit	1.0397	1.0400
Mature seedling grapefruit	1.0380	1.0423

	Calyx quarter	Combined intermediate quarters	Stylar quarter
	sp. gr.	*sp. gr.*	*sp. gr.*
Mature Valencia oranges	1.0434	1.0524	1.0591

The data for the specific gravity at 21° C (using water as standard) of the juice of the different portions of ripe and of green fruit are given in table 1.

The juice samples were obtained in each case from 50 fruits collected at Riverside on November 4, 1932. No significant differences were found in the halves of either lemons or green grapefruit. In both the ripe seedling grapefruit and mature Valencia oranges the specific gravity was greater in the stylar than in the calyx portion.

Frequently the authors observed marked differences in the intensity of the yellow color in numerous samples of centrifuged Valencia orange

juice. Quantitative determinations were made of the carotinoid content of the juice, which was obtained with a hand extractor from the calyx and stylar quarters of pulp.

Fifty cc of centrifuged juice was run into a separatory funnel, 25 cc of chloroform was added, and the whole thoroughly shaken and centrifuged. The yellow pigments which dissolved in the lower chloroform layer were compared in a Dubosq microcolorimeter. When the weaker concentration was taken as unity the carotinoid content of the juice of the stylar quarter was found to be 1.54 times that of the juice of the calyx quarter. In the preparation of orange juice for the market, the reduced concentration of desirable yellow pigments and sugar in the calyx quarter may warrant the discarding of that portion.

Recently Joslyn and Marsh[20] have found that as orange juice deteriorates its iodine-titration value decreases. The writers have determined the iodine-fixation capacity of the juices of the pulp of the calyx and stylar portions of 50 citrus fruits in each case. The juice of the calyx half of mature and immature Valencia oranges and of immature Marsh seedless grapefruit was found to have a greater iodine-fixing power than that of the stylar portion, while the iodine-fixing power of the juice of the stylar portion of mature seedy Marsh grapefruit and of immature lemons and Washington Navel oranges slightly exceeded that of the calyx portion. The iodine-fixation power therefore varies with the degree of maturity, the species, and the portion of the pulp from which it is obtained.

Total and Amino Nitrogen Content of Valencia Orange Juice.—Determinations of total nitrogen, including nitrates (Ranker[25]) were made of the centrifuged juice of calyx and stylar quarters of fruit collected at Riverside on November 19, 1931. Two lots of 50 fruits each supplied the juices for the two sets of determinations. The results are expressed as grams of nitrogen per million cubic centimeters of juice. In the first lot the total nitrogen in the juice of the stylar quarter averaged 1,447 grams and that in the juice of the calyx quarter, 1,508 grams. In the second lot the values obtained were 1,534 and 1,630 grams, respectively. The amino nitrogen content of the juices of both quarters showed no differences: 2 cc of juice contained 1.12216 mg of amino nitrogen at 22° C and 726.7 mm. Copeman[7] has shown that during the ripening of Washington Navel oranges there is no significant increase in the nitrogen or ash content of the juice.

Inorganic Constituents of Juice of Mature Valencia and Navel Oranges.—Copeman[7] found that during the ripening of Washington Navel oranges, calcium and phosphate decreased in the juice while potassium increased. Table 2 shows the gradient in dry matter of the juice

of 50 Valencia oranges. In general the percentage of dry matter increases from the calyx to the stylar end, as was the case with the sugars referred to later in this paper. In contrast, the percentage of total ash of the juice decreases from the calyx to the stylar end. Of the individual

TABLE 2

INORGANIC COMPOSITION, TOTAL SUGARS (AS REDUCING SUGARS), AND REDUCING SUGARS, IN MATURE VALENCIA ORANGE JUICE

(Expressed as Grams per Million cc of Juice)

Section of pulp	Dry matter	Ash	Ca	Mg	K	Na	Inorganic PO_4	Total sugars	Reducing sugars
October 1, 1931									
Calyx half	....	3,920	146	152	1,552	519	668	....	....
Stylar half	....	3,551	120	168	1,282	312	756	....	....
October 6, 1931									
Calyx half	86,708	3,972	204	146	1,634	502	608	....	....
Stylar half	104,643	3,606	138	134	1,329	311	712	....	....
October 14, 1931									
Calyx half	100,630	4,737	152	157	2,112	602	645	....	....
Stylar half	131,473	4,303	130	162	1,836	483	752	....	....
October 15, 1931								October 17, 1931	
Calyx quarter	80,389	5,606	148	180	2,590	737	671	64,176	....
Second quarter from calyx end	106,309	4,731	131	167	2,190	560	632	79,576	....
Third quarter from calyx end	145,939	4,501	98	157	1,900	496	702	96,513	....
Stylar quarter	155,473	4,517	72	152	1,901	448	788	95,680	....
October 23, 1931								October 24, 1931	
Calyx quarter	77,494	4,975	160	163	2,396	641	803	54,996	30,320
Stylar quarter	118,421	4,107	98	168	1,643	430	870	98,013	51,676

ash constituents, calcium, potassium, and sodium increase in concentration from the stylar to the calyx end, while inorganic phosphate increases in the opposite direction. The results show large concentrations of potassium and inorganic phosphate and relatively lower concentrations of calcium and magnesium in the juice. However, from the standpoint of absolute values, the calcium and magnesium concentrations are fairly high and are of approximately the same magnitude.

TABLE 3

Average Inorganic Composition of 50-cc Aliquots of Mature Washington Navel Orange Juice

Section of pulp	Dry matter, in grams	As a per cent of dry matter						As a per cent of ash					Grams per million cc of juice						
		Ca	Mg	K	Na	Inorganic PO_4	Ash	Ca	Mg	K	Na	Inorganic PO_4	Dry matter	Ca	Mg	K	Na	Inorganic PO_4	Ash
Fifty Navel oranges from Riverside, March 8, 1932																			
Calyx quarter	4.6090	0.14	0.18	2.95	0.62	0.87	6.29	2.22	2.75	46.49	9.83	13.71	92,181	129	160	2,690	569	794	5,795
Second quarter from calyx end	5.0655	0.08	0.14	2.14	0.38	0.56	4.68	1.70	3.11	45.08	8.97	11.82	101,309	79	145	2,159	429	566	4,745
Third quarter from calyx end	5.3781	0.06	0.11	1.91	0.42	0.57	4.11	1.60	2.77	45.07	9.33	13.41	107,561	67	116	2,048	425	609	4,423
Stylar quarter	5.8054	....	0.12	1.93	0.42	0.64	4.17	1.47	2.77	45.96	10.08	15.52	116,107	72	136	2,198	483	744	4,839
Fifty extremely large jumbo Navel oranges from young trees at Hemet, January 21, 1932																			
Calyx quarter	5.7954	....	....	2.47	0.57	....	5.30	2.48	2.44	47.24	10.94	9.60	115,948	154	152	2,863	663	595	6,148
Stylar quarter	6.0204	....	....	2.00	0.43	....	4.36	1.73	2.49	45.74	9.90	12.83	120,408	91	131	2,403	520	672	5,248
Secondary fruit	5.9186	....	....	2.07	0.49	....	4.53	1.03	2.27	44.97	11.77	15.86	118,372	55	122	2,415	527	853	5,372

Table 3 gives the results of similar analyses of the pulp juice of mature Washington Navel oranges of commercial size, and also of extremely large jumbo Washington Navel oranges and of their small, secondary fruit found at the navel end. The dry matter of the juice of the pulp of oranges of commercial size shows an increase from the calyx to the stylar end as in Valencia orange juice. The percentages of calcium, magnesium, sodium, potassium, and total ash in the dry matter increase from the stylar to the calyx end. Inorganic phosphate is higher at the calyx and stylar ends than in the middle portion.

The grams of dry matter in 1,000,000 cc of juice show an increase from the calyx to the stylar end, and the grams of ash from the center toward both ends. Based on total ash, the percentages of calcium increase from the stylar toward the calyx end.

The results shown in table 3 for the juices of 50 extremely large Washington Navel oranges and of their small, secondary fruits are somewhat different from those obtained from commercial fruits. The concentrations of the dry matter in the juices of the various portions of the pulp are similar and are relatively high. The concentrations of ash, sodium, and potassium in the dry matter of the juice of the secondary fruit more nearly resemble those of the stylar end than those of the calyx end. The concentrations of calcium and magnesium in the juice show an increase from the secondary fruit to the calyx end, and those of potassium from the stylar to the calyx end. The increase in inorganic phosphate is from the calyx to the secondary fruit which is in the same direction as that found in the Valencia orange.

Total and Reducing Sugars in Valencia Orange Juice.—Total sugars were determined as reducing sugars after HCl hydrolysis, by use of the Shaffer and Hartmann[27] iodometric method. The juice was secured from the calyx and stylar halves of each of three lots of 50 mature Valencia oranges which were secured at Riverside (two lots on October 6, 1931, and a third lot on October 15, 1931). For the calyx half, the average total sugar content of the three lots was 73,567 grams per million cc of juice, and for the stylar half, 94,133 grams. As will be discussed later, this is one of the chief factors that account for the lower loss of water from the stylar portion of oranges.

The sugar content of the juice of mature Valencia oranges varies, according to the age of the fruit, the health of the tree, and other factors. Table 2 gives more complete data on the gradient in total and reducing sugars of the juice of the various portions of the pulp. These data were secured from two lots each consisting of 50 fruits obtained at Riverside. The determinations were made respectively on the juice of the calyx, stylar, and two middle quarters of the pulp and show a concentration in-

crease from the calyx to the stylar portion. This sugar gradient is so striking as to be easily recognized by taste.

Total Sugars in Juice of Lemons and Grapefruit.—Determinations were made of the total sugars in the juice of lemons in the silver stage, Marsh grapefruits which were nearly full-sized, but green, and overmature seedling grapefruits obtained at Riverside on November 2, 1932.

TABLE 4

TOTAL SUGARS, AS DEXTROSE IN JUICE OF LEMONS AND GRAPEFRUIT

Type of fruit	Number of fruits	Grams sugar per million cc in calyx halves	Grams sugar per million cc in stylar halves
Silver-stage lemons	42	26,580	28,365
Green Marsh grapefruits	26	55,780	58,025
Overripe seedling grapefruits	25	70,810	79,870

In every case (table 4) there were more total sugars in the juice of the stylar half than in the calyx half, although the differences were not as large as those in Valencia oranges (table 2).

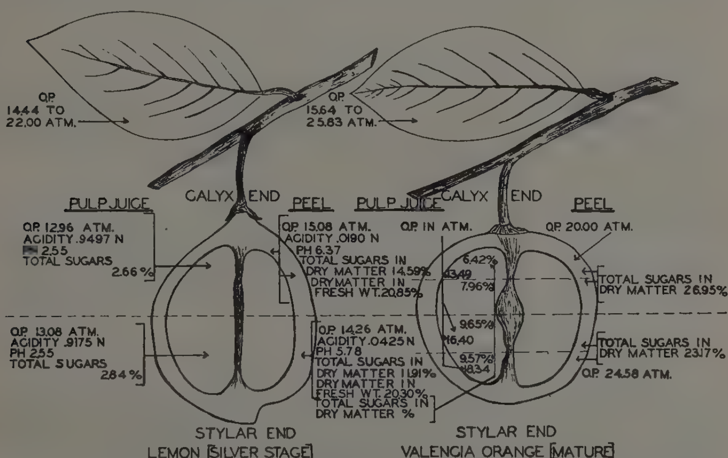


Fig. 1.—Acidity, dry matter, total sugars, and osmotic pressure in different portions of lemon and orange fruit and osmotic pressure of leaf sap.

Osmotic Pressure.—Osmotic pressure of pulp juice of lemons (silver stage) and of mature Valencia oranges was determined as described in "Methods of Analysis." Figure 1 shows that the juice of the calyx half of the lemon pulp had an osmotic pressure of 12.96 atmospheres and that of the stylar half 13.08 atmospheres. The juices of Valencia orange showed far greater differences in osmotic pressure than those of

lemon. Figure 1 indicates that the juice of the calyx quarter had an osmotic pressure of 13.49 atmospheres, that of the middle half 16.40 atmospheres, and that of the stylar quarter 18.34 atmospheres. The variations in osmotic pressure were largely the result of the differences in the sugar content of the juice of different portions of the pulp. This is evident from table 2, which shows the greatest inorganic content in the calyx portion. The significance of the gradient in osmotic pressure will be discussed later.

TABLE 5

Inorganic Composition of Peel and Pulp of Mature Lemons and Grapefruit

Portion of fruit	Per cent of dry matter in fresh weight	As a per cent of dry matter					
		Ash	Ca	Mg	Na	K	Inorganic PO_4
Twenty-six Eureka lemons, September 5, 1930							
Calyx half of peel	20.85	3.75	1.15	0.14	0.09	0.39	0.15
Stylar half of peel	20.30	3.69	1.02	0.10	0.05	0.58	0.19
Twelve Eureka lemons, March 22, 1931							
Peel	....	4.94	1.26	0.13	0.18	0.92	0.28
Pulp, no seed	....	4.61	0.38	0.12	0.45	1.92	0.54
Peel and pulp, no seed	....	5.31	0.98	0.15	0.33	1.39	0.42
Twenty-five Marsh grapefruits, June 17, 1932							
Calyx half of peel	15.78	3.14	0.63	0.10	0.15	0.82	0.25
Stylar half of peel	17.08	2.89	0.53	0.08	0.13	0.81	0.33
Calyx half of pulp	8.39	3.52	0.28	0.12	0.28	1.42	0.64
Stylar half of pulp	8.98	3.22	0.21	0.11	0.27	1.26	0.70

GRADIENTS IN COMPOSITION OF CITRUS PULP

Inorganic Constituents of Pulp of Lemons, Grapefruit, and Valencia Oranges.—The inorganic composition of the pulp of lemons and of grapefruit is shown in table 5. The percentages of calcium and potassium of the calyx halves of pulp of grapefruit were greater than those of the stylar halves while the phosphate was greater in the stylar halves.

Data are given in table 6 for the inorganic composition of the calyx and stylar halves of pulp of 16 Valencia oranges. Even though the percentage of dry matter is greater in the stylar half, the percentages of ash, calcium, magnesium, potassium, and sodium are greater in the dry matter of the calyx half. The percentages of calcium, potassium, and sodium are higher in the ash of the calyx half, while those of magnesium

and inorganic phosphate are greater in that of the stylar half. When the peel is included with the pulp, the results are not appreciably altered.

The percentages of total sulfur and phosphorus were determined in the dry matter of the seed-free pulp of mature Valencia oranges collected in San Diego County. The total sulfur content was found to be 0.072 per cent, and the total phosphorus content 0.129 per cent.

Additional data are given in table 6 on the pulp of Valencia oranges from Riverside County. These oranges were prepared for analysis immediately after being picked and were not coated with paraffin as were those used in another experiment (table 6). On a dry-matter basis the percentages of ash, calcium, potassium, and sodium are greater in the calyx half, while the percentage of inorganic phosphate is greater in the stylar half.

The analysis of the pulp of 79 Valencia orange fruits collected on the same day in Orange County showed for the ash constituents in the dry matter gradients similar to those found in the fruits from Riverside County. The percentages of the several ash constituents of the fruit pulp from both locations were also similar, with the exception that the ash content of the Riverside-County fruit was 20 per cent greater than that from Orange County. This was due largely to the greater amounts of potassium, phosphate, and sodium in the Riverside-County fruit. In the calyx and stylar halves of the dry pulp of Orange-County fruit, the respective percentages were 1.46 and 1.16 for potassium; 0.39 and 0.24 for sodium; and 0.550 and 0.580 for inorganic phosphate. These percentages may be compared with those for the Riverside-County fruit given in table 6. The reasons for the differences between the fruits from the two locations are at present unknown.

Equatorial Radial Gradient in Ash of Valencia Oranges.—Two lots each consisting of 20 mature Valencia oranges obtained on June 29, 1932, were peeled and the calyx and stylar quarters of the pulp were cut away and discarded. The central core and the outer membrane of the pulp were removed. The pulp was separated into an inner and outer portion by cutting parallel to the core and making an inner cylinder with a radius equal to about two-thirds of the radius of the pulp. After the seeds were removed the two portions of pulp were dried to constant weight and ashed. The percentages of ash in two samples of the dry matter of the central portion were 4.06 and 3.94, while those for two samples of the outer portion were 2.92 and 2.96. An explanation of the direction of this ash gradient toward the central core is found in the arrangement of the juice vesicles in the pulp. The outer layers of the carpels are united along the whole length of their edges to form the rind, while the inner layers fold toward the core of the fruit to form the seg-

TABLE 6

INORGANIC COMPOSITION OF PEEL AND PULP OF VALENCIA ORANGES FROM RIVERSIDE, 1931

Portion of orange	Fresh weight, in grams	Per cent of dry matter in fresh weight	As a per cent of dry matter: Ash	Ca	Mg	K	Na	Inorganic PO_4	As p.p.m. of dry matter: Fe	Mn
One hundred fruits, August 5										
Calyx quarter of peel	1,030.5	19.46	5.02	1.01	0.16	1.26	0.29	0.240	77	5.8
Second quarter of peel from calyx end	1,013.5	22.60	4.28	0.70	0.11	1.31	0.32	0.265	....	5.3
Third quarter of peel from calyx end	1,025.5	25.30	3.92	0.61	0.09	1.24	0.27	0.300	96	5.0
Stylar quarter of peel	804.5	27.72	3.87	0.59	0.08	1.19	0.26	0.365	....	6.1
Calyx half of pulp	2,790.5*	11.65	4.56	0.32	0.13	1.94	0.45	0.620	....	3.6
	2,779.5*	11.46	4.52	0.32	0.13	1.89	0.47	0.645	....	3.7
Stylar half of pulp	2,760.5*	13.11	3.65	0.24	0.12	1.42	0.37	0.670	....	3.3
	2,782.5*	13.01	3.70	0.23	0.12	1.47	0.36	0.675	....	4.2
Fourteen fruits, August 20										
Calyx half of peel and pulp	917.0	14.94	3.74	0.51	0.14	1.18	0.16	0.500	....	....
Stylar half of peel and pulp	913.0	16.87	3.15	0.39	0.11	1.06	0.13	0.550	....	....
Group A. Sixteen fruits, air-dried from August 20 to October 10. First lot of fruit, with calyx half of peel paraffined on August 20										
Calyx half of peel	....	33.36†	....	....	....	....	....	....	....	....
Stylar half of peel	222.0†	28.38	3.65	0.73	0.10	0.89	0.17	0.410	117	....
Calyx half of pulp	797.5†	10.60	4.24	0.31	0.14	1.75	0.41	0.800	252	....
Stylar half of pulp	833.0†	12.42	3.32	0.22	0.12	1.23	0.26	0.750	....	3.2

* Sample consisted of pulp of 50 fruits.

† After air-drying.

TABLE 6—*(Continued)*

Portion of orange	Fresh weight, in grams	Per cent of dry matter in fresh weight	As a per cent of dry matter						As p.p.m. of dry matter	
			Ash	Ca	Mg	K	Na	Inorganic PO_4	Fe	Mn
Second lot of fruit of Group A, with stylar half of peel paraffined on August 20										
Calyx half of peel	186.5†	30.83	4.08	1.00	0.12	0.69	0.12	0.280	362	....
Stylar half of peel	....	35.81†	....	....	....	....	....	....	....	....
Calyx half of pulp	724.5†	11.15	4.32	0.36	0.14	1.68	0.44	0.655	....	....
Stylar half of pulp	779.0†	12.58	3.44	0.27	0.12	1.32	0.29	0.740	61	....
Group B. Sixteen fruits, air-dried from August 20 to October 10. First lot of fruit, with calyx half of peel paraffined on August 20										
Calyx half of peel	....	32.21†	....	....	....	....	....	....	....	....
Stylar half of peel	245.5†	30.35	3.61	0.72	0.10	0.86	0.17	0.400	149	....
Calyx half of pulp	857.0†	12.72	3.75	0.31	0.08	1.49	0.33	0.690	79	....
Stylar half of pulp	834.0†	14.57	3.05	0.24	0.10	1.17	0.22	0.680	....	....
Second lot of fruit of group B with stylar half of peel paraffined on August 20										
Calyx half of peel	191.0†	31.28	4.16	0.87	0.14	0.84	0.16	0.320	192	....
Stylar half of peel	....	36.53†	....	....	....	....	....	....	....	....
Calyx half of pulp	768.0	12 96	3.80	0 32	0.12	1.50	0 35	0.660	34	3.6
Stylar half of pulp	800.5	14.43	2.98	0.25	0.11	1.12	0.27	0.600	....	3.1

† After air-drying.

ments, including their separating membranes. The juice vesicles arise as modified trichomes on the inner surface of the mesocarp, their free terminals extending toward the central core. The inside portion of pulp therefore consists largely of the termini of the juice sacs in which, as the analyses show, the ash accumulates somewhat as in the tip of a leaf.

Inorganic Constituents of Calyx Ends of Granulated and Control Valencia Oranges.—In the observations made late in November, 1931, it was frequently found that portions of the pulp of the calyx end were

TABLE 7

INORGANIC CONTENT OF GRANULATED AND CONTROL CALYX-END EIGHTHS OF VALENCIA ORANGE PULP (NO PEEL OR CORE), NOVEMBER 25, 1931

Number of eighths	Fresh weight, in grams	Dry matter as per cent of fresh weight	As a per cent of dry matter						As parts per million of dry matter	
			Ash	Ca	Mg	K	Na	Inorganic PO_4	Fe	Mn
92 (control)	1,174.0	10.78	5.16	0.38	0.15	2.12	0.45	0.85	81	4.0
145 (granulated)	1,541.5	9.89	6.13	0.45	0.11	2.55	0.61	0.84	71	5.3

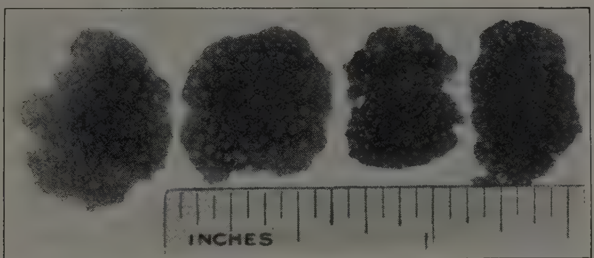
severely granulated. A large number of such affected calyx-end portions were compared chemically with a similar number of unaffected calyx-end portions. The results given in table 7 show that the affected pulp contained the higher percentages of water. The percentages of ash, calcium, potassium, and sodium were higher in the dry matter of the granulated pulp, while those for magnesium and iron were lower. When calculated as percentages of the ash, the granulated pulp contained the smaller percentages of calcium, magnesium, and inorganic phosphate. If we assume that the percentages of ash in the unaffected stylar pulp of granulated and of control fruits are similar, it is evident that a steeper gradient exists in granulated fruit.

Total Sugars of Grapefruit.—Determinations of the total sugars were made with the dried pulp of the calyx and stylar halves of 25 Marsh grapefruits collected at Riverside on June 17, 1932. The seeds were removed in the preparation of the samples. The fresh and dry weights of the calyx pulp were 3,518 and 295.25 grams, respectively, which is equivalent to 8.39 per cent of dry matter, 10.87 per cent of the total dry matter being sugars. The fresh and dry weights of the stylar pulp were 3,420 and 307 grams, respectively, corresponding to 8.98 per cent of dry matter, 13.12 per cent of the total dry matter being sugars.

GRADIENTS IN COMPOSITION OF CITRUS PEEL

Oil Content of Calyx and Stylar Ends.—Determinations of the oil content of calyx and stylar disks of equal surface area of Valencia orange peel were made on September 25, 1931, as described under "Methods of Analysis." The results showed a greater amount of oil in the stylar portion.

In each case the white portion of seven disks was cut back to make the calyx and stylar peel of equal thickness. The average results of four



A *B* *C* *D*

Fig. 2.—Oil glands in Valencia orange peel. *A*, Outside surface of calyx end; *B*, inside surface of calyx end; *C*, inside surface of stylar end; *D*, outside surface of stylar end.

closely agreeing determinations were for the calyx and stylar ends, respectively, 0.1103 and 0.1699 grams of oil.

In another analysis, using steam distillation, oil was extracted from 70 disks of equal surface area of the calyx and stylar peel. The weight of oil residue found for the calyx peel was 0.0110 gram, while that for the stylar peel was 0.0142 gram. Figure 2 shows the difference in number and size of oil-bearing glands when thin slices of orange peel were photographed by transmitted light.

Inorganic Constituents.—When fruit was exposed on a table from August 18 to September 1, 1931, the gradient for the dry matter in the quarters of peel was still evident, but because of the desiccation the percentages were on a higher level. Fruit with leafy twigs attached and exposed similarly, showed a similar gradient but on a slightly higher level. However, it will be noted that little additional water was lost on account of the leafy twigs.

The results of the inorganic analysis of Valencia orange peel given in table 8 show a gradient in the percentages of dry matter. The percentages of ash, calcium, magnesium, and iron in the dry matter increase

TABLE 8

DRY MATTER AND INORGANIC COMPOSITION OF PEEL AND PULP OF LOTS OF 26 VALENCIA ORANGES FROM RIVERSIDE, AUGUST 18, 1931

	Fresh weight, in grams	Per cent of water	Per cent of dry matter in fresh weight	As a per cent of dry matter						In dry matter, p.p.m.	
				Ash	Ca	Mg	K	Na	Inorganic PO_4	Fe	Mn
Control fruit prepared for analysis immediately after being picked*											
Calyx quarter of peel	270.00	78.33	21.67	3.85	1.06	0.170	0.43	0.08	0.22	192	......
Second quarter of peel from calyx end	253.50	73.77	26.23	3.11	0.79	0.110	0.49	0.10	0.26	122	5.0
Third quarter of peel from calyx end	284.50	70.30	29.70	2.95	0.72	0.090	0.57	0.14	0.33	......	......
Stylar quarter of peel	205.00	69.27	30.73	2.98	0.69	0.090	0.61	0.14	0.38	......	5.2
Calyx half of pulp	1,394.00	87.37	12.63	3.37	0.32	0.108	1.25	0.30	0.60	......	......
Stylar half of pulp	1,383.50	85.15	14.85	2.65	0.24	0.091	0.91	0.25	0.60	......	......
Fruit without leafy twigs attached; exposed August 18 to September 1 at room temperatures†											
Calyx quarter of peel	227.00	68.94	31.06	3.72	1.01	0.162	0.52	0.11	0.24	......	6.0
Second quarter of peel from calyx end	221.00	68.78	31.22	3.13	0.76	0.100	0.53	0.09	0.28	160	......
Third quarter of peel from calyx end	213.00	67.84	32.16	3.06	0.72	0.090	0.60	0.13	0.32	156	......
Stylar quarter of peel	211.00	66.82	33.18	3.12	0.66	0.090	0.67	0.16	0.42	......	5.0
Calyx half of pulp	1,496.00	89.37	10.63	3.99	0.36	0.126	1.46	0.38	0.71	......	3.2
Stylar half of pulp	1,521.00	88.20	11.80	3.17	0.26	0.096	1.13	0.22	0.73	......	2.7
Fruit with leafy twigs attached; exposed August 18 to September 1 at room temperatures‡											
Calyx quarter of peel	186.00	65.86	34.14	3.94	1.04	0.180	0.44	0.11	0.25	162	......
Second quarter of peel from calyx end	192.00	65.36	34.64	3.26	0.80	0.120	0.53	0.08	0.30	149	......
Third quarter of peel from calyx end	193.00	64.25	35.75	3.20	0.76	0.100	0.63	0.10	0.36	107	......
Stylar quarter of peel	158.00	63.29	36.71	3.32	0.74	0.100	0.67	0.12	0.47	142	......
Calyx half of pulp	1,215.00	88.15	11.85	3.79	0.39	0.120	1.39	0.26	0.67	......	3.5
Stylar half of pulp	1,310.00	85.91	14.09	2.98	0.30	0.100	1.10	0.21	0.66	38	3.0

* Fresh weight, 3,832 grams.
† Fresh weight on August 18, 4,322 grams; fresh weight on September 1, 3,927 grams. Fresh and dry weights in table as of September 1.
‡ Fresh weight on September 1, 3,299 grams. Fresh and dry weights in table as of September 1.

toward the calyx end, while those for sodium, potassium, and inorganic phosphate increase toward the stylar end. When the results are expressed as per cent of ash, the directions of the gradients remain unchanged.

The percentages of total sulfur and phosphorus were determined in the dry matter of the peel of mature Valencia oranges collected later in San Diego County, the data of which are not shown in table 8. For the stem half the total sulfur was found to be 0.038 per cent and for the tip half 0.052 per cent. In the case of total phosphorus the percentages were 0.093 and 0.095, respectively.

The results for the peel of calyx and stylar halves of lemons collected at Riverside September 5, 1930, show in table 5 that the percentages of ash, calcium, magnesium, and sodium in the dry matter are greater in the calyx half, while the percentages of potassium are greater in the stylar half. The gradients of the inorganic constituents are therefore in a direction similar to those obtained for the peel of Valencia oranges (tables 6 and 8).

The percentages (table 5) of calcium in the dry matter and ash of the peel of lemons collected on March 22, 1931, at Riverside, exceed those found in the pulp, while those of potassium and inorganic phosphate are less than those found in the pulp. The percentages of calcium, magnesium, and sodium are greater in the dry matter or ash of the calyx halves while those of potassium and phosphate are greater in the dry matter or ash of the stylar halves. The directions of gradients of the inorganic constituents of the dry matter and ash are the same as those for Valencia orange (table 8).

Results with the calyx and stylar halves of peel of grapefruit collected June 17, 1932, at Riverside, show (table 5) that the percentage of dry matter is greater in the stylar half, as was found in Valencia oranges. Except for the potassium in the dry matter, the percentages of inorganic constituents in the dry matter and ash are in the same direction as in lemons and Valencia oranges.

Table 6 shows gradients in the inorganic constituents of the peel of Valencia oranges collected at Riverside on August 5. Similar determinations were made on 79 Valencia oranges collected on the same day in Orange County, the results for which showed similar gradients. The percentages of ash, potassium, and sodium in the peel of Orange-County fruits, however, were much less than those in the Riverside fruit, being 3.23 to 3.79 for ash, 0.65 to 0.80 for potassium, and 0.08 to 0.18 for sodium.

Radial and Polar Gradients of Inorganic Constituents of Valencia Oranges.—Since orange peel is thickest at the calyx end, it was divided

not only into calyx and stylar portions, but also radially into the gland-bearing (outer) and white (inner) portions. Determinations were made of the inorganic composition of these various portions of peel of two lots each consisting of 20 Valencia oranges collected from the fertilizer plots at the Citrus Experiment Station on June 29, 1932. Lot B was secured from a plot that received urea but no potassium or covercrop.

TABLE 9

INORGANIC COMPOSITION OF GLAND-BEARING (OUTER) AND WHITE (INNER) PORTIONS OF LOTS OF 20 CALYX AND STYLAR HALVES OF VALENCIA ORANGE PEEL

Portion of peel	As a per cent of dry matter					
	Ash	Ca	Mg	Na	K	Inorganic PO_4
Lot A						
Outer portion of calyx half	4.19	0.98	0.18	0.11	0.76	0.35
Inner portion of calyx half	3.05	0.90	0.67	0.02	0.34	0.20
Outer portion of stylar half	3.67	0.77	0.12	0.10	0.86	0.48
Inner portion of stylar half	2.72	0.81	0.06	0.02	0.36	0.18
Lot B						
Outer portion of calyx half	3.71	0.95	0.18	0.09	0.56	0.28
Inner portion of calyx half	2.53	0.76	0.67	0.02	0.29	0.11
Outer portion of stylar half	3.42	0.75	0.11	0.15	0.77	0.45
Inner portion of stylar half	2.39	0.67	0.07	0.03	0.36	0.17

Lot A was from a plot that received urea and sulfate of potash, but no covercrop. Since potash is the variable in the two plots it is possible that differences in composition of the fruit may be related to the potash fertilization of the soil.

In both lots of fruit (table 9) the gland-bearing portion contained a greater percentage of ash than the white portion, and the values for the calyx half were greater than those for the stylar. Moreover, the percentage of ash in the dry matter was greatest in lot A.

In both lots of fruit the percentage of calcium in the dry matter was greater in the calyx than in the stylar half, and in most cases was greater in the gland-bearing portion of the peel than in the white portion. In the fruit examined, the percentages of calcium in the dry matter were larger in lot A.

The percentages of magnesium in the dry matter were approximately the same in lots A and B. It should be mentioned here that in the calyx

half there was nearly four times as great a percentage of magnesium in the dry matter of the white portion as in the gland-bearing portion, and in the stylar half there was only twice as great a percentage.

In every case a larger percentage of potassium was found in the dry matter of the gland-bearing portion, and there was a larger percentage in the gland-bearing portion of the stylar half than in that of the calyx half. The greater percentages of potassium in the dry matter of the peel of the stylar half confirm previous results (table 8). Since it has been shown that the percentages of sugars are less in the dry matter of the peel of the stylar half, it appears that on a dry-matter basis the percentages of potassium bear no positive relation to the sugar content. However, there was a positive relation of inorganic phosphate and nitrogen to sugar.

The percentages of potassium in the gland-bearing portion were highest and varied the most, while those of potassium in the white portion were relatively constant. In fruit from lot A the percentages of ash in the gland-bearing portion of the peel were much higher than in fruit from lot B.

In every case the percentages of inorganic phosphate were greatest in the gland-bearing portion, in which portion the percentages were higher for the stylar than for the calyx end.

The percentages of calcium in the ash vary but little, and are higher in the white portion of the peel. In the gland-bearing portion they are higher in the calyx than in the stylar half. The percentages of magnesium and sodium in the ash are greater in the gland-bearing portion.

The percentages of potassium in the ash are greater in the gland-bearing portion of the peel. The gland-bearing and white portions have higher percentages in the stylar than in the calyx half.

It has been shown that the gland-bearing portion of the calyx half of Valencia-orange peel contains a smaller percentage of potassium than that of the stylar half (table 9). Valencia orange fruits obtained from trees grown in sand cultures that received a culture solution lacking potassium, showed a breakdown and slight gumming in the peel of the calyx half (fig. 3). The outer portion of the peel appeared to be the only part affected. This is of interest because of the frequent assertion that potash improves among other things the quality of the rind.

In these studies of gradients the greatest concentration of potassium usually was not found where the concentration of sugars was greatest. While the role of potassium in the peel, pulp, leaf, or trunk is but little understood, it has been suggested that potassium is concerned with the synthesis of carbohydrates and proteins, although some investigators have found no such relation (Janssen and Bartholomew[19]). Taka-

hashi[28] has maintained that potash fertilization increased the sugar content of citrus fruit. However, for prunes, Lilleland[22] found that although potash fertilization of the soil increased the potassium con-

Fig. 3.—Effect of a deficiency of potassium in sand cultures on peel of Valencia orange. Upper row: magnesium substituted for potassium in culture solution. Lower row: left, calcium substituted for potassium in culture solution; right, control (complete culture solution).

tent of the leaves and fruit, there was no increase in the sugar content of the fruit.

Total Sugar and Starch Content of Calyx and Stylar Halves.—The peel of the calyx halves of 26 Eureka lemons collected September 5, 1930, at Riverside, contained 20.85 per cent dry matter and 14.59 per cent total sugars in the dry matter, while the peel of the stylar halves contained 20.30 and 11.91 per cent dry matter and total sugars in the dry matter, respectively.

Similar determinations were made with grapefruit and Valencia-orange peel. The values obtained for the calyx halves of peel of 25 Marsh grapefruits collected at Riverside were 15.78 per cent dry matter and 28.77 per cent total sugars in dry matter, and for the stylar halves were 17.08 and 27.89 per cent dry matter and total sugars in dry matter.

Determinations of total sugars in the dried peel of two lots of Valencia oranges collected July 20, 1931, at Riverside, gave 25.74 and 28.16 per cent, respectively, for the calyx halves, and 22.66 and 23.67 per cent for the stylar halves.

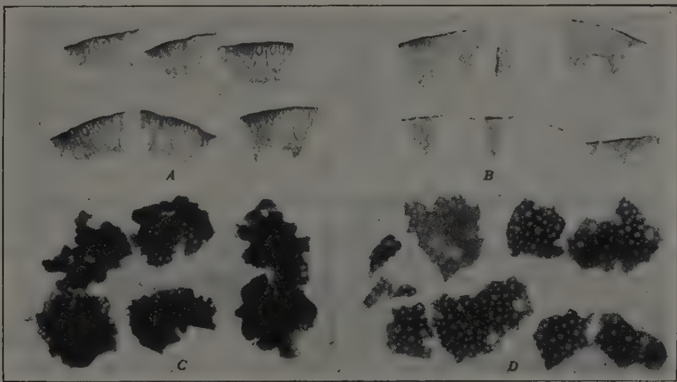


Fig. 4.—Starch in mature Valencia orange peel. *A*, Tip end, radial section; *B*, stem end, radial section; *C*, tip end, tangential section; *D*, stem end, tangential section.

On other samples of dry matter prepared from disks of equal surface area cut from the calyx and stylar ends of the peel of Valencia oranges collected on October 14, 1931, at Riverside, determinations of total sugars showed 25.21 and 24.61 per cent, respectively.

The relative amounts of starch in the calyx and stylar peel of mature Valencia oranges obtained at Riverside on October 15, 1931, may be seen in figure 4, in which fresh, thin sections were stained for equal periods in a solution of iodine and potassium iodide. The reaction for starch appears stronger in the stylar peel. The concentration gradients for sugars and starch in the peel, while small, are the reverse in direction of those for sugars in the pulp.

Osmotic Pressure.—The osmotic pressures of the juices of lemon peel were obtained as described under "Methods of Analysis." Figure 1 shows the osmotic pressure of the juice of the peel from the calyx halves of 50 lemons (silver stage) as being 15.08 atmospheres and that for the juice of the peel from the stylar halves as being 14.26 atmospheres.

The values obtained for the osmotic pressure of the juice of the peel from the calyx and stylar halves of 50 ripe Valencia oranges are shown in figure 1. The value for the calyx halves was 20.00 atmospheres and that for the stylar halves was 24.58 atmospheres. The increased osmotic pressure in the stylar halves cannot be ascribed to an increased percentage of total sugars, because the percentage of total sugars is slightly less in the stylar peel. From the data in table 8 it appears likely that the increased osmotic pressure may be a result of differences in the water content of the halves of peel.

WATER CONTENT OF VARIOUS PORTIONS OF CITRUS FRUITS

Determinations were also made of the water content and dry matter of disks of equal diameter cut from the stem and stylar quarters of mature Valencia orange rind collected September 25, 1931. A sufficient amount of the white (inner) portion was cut from the thicker disks of the calyx end to make them approximate the thickness of those from the stylar end. The 12 disks of the calyx end gave a fresh weight of 10.2006 grams; and dry matter 20.70 per cent of the fresh weight, whereas the 12 disks of the stylar end gave a fresh weight of 8.6170 grams; and dry matter 28.69 per cent of the fresh weight. Thus it is evident that the disks of the calyx end had a greater fresh weight, yet contained less dry matter than those of the stylar end. Further confirmation of these results is reported in table 10.

TABLE 10

WATER CONTENT OF VALENCIA ORANGE PEEL*

Number of disks	Half	Fresh weight, grams	Dry weight, grams, 100° C	Water as per cent of fresh weight
200	Calyx	178.50	38.31	78.54
200	Stylar	125.25	37.94	69.71
335	Calyx	269.07	50.08	81.39
335	Stylar	197.63	55.18	72.08

* Disks of equal surface area (11/16-inch diameter) and natural thickness.

The loss of volatile oils from the rind during heating complicates these determinations so that the values must be regarded as only relative and not absolute. The rind of a given surface of the calyx end is thicker and weighs considerably more than that of a similar surface of the stylar end, yet they closely approximate each other in dry matter per unit of surface. The extra thickness of the peel of the calyx end may be due principally to the swelling that results from its greater power to imbibe water.

The writers have shown that more water is lost through the rind of the calyx end of Valencia oranges than through that of the stylar end, and later in this paper will report further data in partial explanation of these differences.

The water content of the calyx half of mature Valencia orange pulp is shown in tables 6 and 8 to exceed that of the stylar pulp.

The uniformity of the water content of the various portions of lemon peel is seen in table 5.

Determinations of the water content of the two halves of 120 freshly picked tree-ripe lemons showed 84.41 per cent in the calyx halves and 84.0 per cent in the stylar halves. Since the fruit was picked shortly following a 2-day period of heavy rain, the trees should not have been suffering from a water deficit. Similar results were obtained by Bartholomew[(1)] with determinations made in January, February, and March.

In the case of grapefruit peel and pulp the calyx half contains more water than the stylar half (table 5). Thus the water content of grapefruit more nearly parallels that of the orange than the lemon.

GRADIENTS IN PERMEABILITY OF CALYX AND STYLAR HALVES OF CITRUS

It has been shown that the calyx half of lemons and Valencia oranges loses water more rapidly than the stylar half, and it was therefore of interest to study the loss of other constituents from the fruit. This was accomplished by measuring the rate of decolorization of the $KMnO_4$ by materials that exosmose from fruit into water.

A comparison was made of the relative permeability of calyx and stylar halves of green lemons, green Marsh grapefruit, and Valencia oranges. The lots were paraffined as described under "Methods of Analysis" and placed in 2,000 cc of tap water. The data are given in table 11.

The data show that the differences in permeability of the halves of green lemons are small. The calyx half shows only a slightly greater permeability. These results are in agreement with the observations that the various portions of the peel differ but little in their susceptibility to storage breakdowns and fungus invasion.

The results for grapefruit and Valencia oranges show that the calyx half has a distinctly greater permeability than the stylar half, which is in agreement with the loss of water by evaporation from the halves of Valencia oranges.

Since the permeability of citrus fruit increases with increasing age (Klotz[(21)]) and since the calyx half is more permeable than the stylar

half, the indications are that the calyx portion has aged more rapidly. It is known, moreover, that the calyx half is more subject to certain diseases and physiological breakdowns than the stylar half. The importance of methods of studying changes in the permeability of different portions as well as in whole fruit becomes greater where such changes can be related to other gradients.

TABLE 11

TIME REQUIRED TO MATCH $KMnO_4$ STANDARD IN PERMEABILITY TESTS OF CALYX AND STYLAR HALVES OF LEMONS, GRAPEFRUIT, AND ORANGES

Treatment of fruit halves	Time immersed, hours	Minutes and seconds required to match standard				
Green lemons from Riverside, October 28, 1932; lots of 20 fruits each, three determinations						
Calyx half not paraffined	16	15:23	12:22	....	....	....
Stylar half not paraffined	16	15:23	12:22	....	....	....
Calyx half not paraffined	66	1:80	1:50	1:30	....	....
Stylar half not paraffined	66	1:42	1:40	1:55	....	....
Nearly full-grown green Marsh grapefruit from Riverside, November 1, 1932; lots of 10 fruits each, four determinations						
Calyx half not paraffined	48	3:16	2:10	3:00	3:15	....
Stylar half not paraffiined	48	9:46	11:10	10:00	9:51	....
Valencia oranges from Riverside, October 27, 1932; lots of 20 fruits each, five determinations						
Calyx half not paraffined	....	1:52	2:15	2:00	2:05	1:55
Stylar half not paraffined	....	7:12	5:00	7:20	7:40	7:20

The method for estimating permeability was used in a preliminary experiment to determine the effect of hydrocyanic acid gas on lemon fruit. Ten (300 size) silver-stage lemons were obtained directly from the trees at Riverside and were fumigated with 15 cc HCN in a chamber (100 cubic feet) for 35 minutes. This and the control fruit were placed in tap water at 20° to 21° C for 16 hours. Aliquots of the solution containing the fumigated fruit required 4 minutes to decolorize the $KMnO_4$, while aliquots of the solution containing the control fruit required 11½ minutes. This suggests that the permeability of the peel of lemons is increased by the process of fumigation.

The relative effect of hydrocyanic acid gas on the permeability of the two halves of Valencia oranges was next determined. Ten fruits collected March 25 were used in each of four lots. The half not under consideration was paraffined before exposure to the gas. In each case the fruits were immersed in 2 liters of distilled water for 22 hours, and the

rate of $KMnO_4$ decolorization was estimated and compared with control fruit similarly paraffined but not fumigated. The time required (hours and minutes) to match the $KMnO_4$ standard was as shown in the following tabulation:

Calyx half not paraffined; stylar half paraffined; fruit fumigated	1:15
Calyx half not paraffined; stylar half paraffined; fruit not fumigated (control)	11:05
Stylar half not paraffined; calyx half paraffined; fruit fumigated	3:25
Stylar half not paraffined; calyx half paraffined; fruit not fumigated (control)	14:30

The data again show that the calyx half of the unfumigated (control) fruit had a greater permeability than the stylar half. The effect, however, of the hydrocyanic acid treatment was not only to increase the permeability of both halves but also to increase that of the calyx half more than that of the stylar half. The results correspond with the degree of injury from hydrocyanic acid treatment of Valencia oranges in the field where the calyx half is ordinarily the first portion to be injured.

TABLE 12

WATER LOSS THROUGH PEEL OF HALVED FRUITS WITH CUT SURFACE ON PARAFFINED PAPER, 1930

Half	Number of fruits	Water loss after 5 days as a per cent of original weight	Water loss after 8 days as a per cent of original weight
Mature Valencia oranges			
Calyx	31	4.80	12.90
Stylar	31	3.88	9.74
Lemons (silver stage)			
Calyx	48	9.34	13.32
Stylar	48	7.40	12.21

GRADIENTS IN WATER LOSS FROM CITRUS FRUITS

A knowledge of the water relations of leaves, twigs, and portions of the fruit may lead to a better understanding of the interrelations of organic and inorganic substances within these organs. The physiological diseases called granulation and endoxerosis have been considered in connection with the water relations of the fruit, especially with the gradient existing between the calyx and the stylar ends. At the initiation of this work, the writers investigated the source, causes, and pre-

vention of water loss in fruit, and the bearing of tissue structure and composition upon these factors.

Permeability of Peel.—Water losses through the peel of halved lemons and Valencia oranges were first determined, without the use of boric acid antiseptic on the cut surfaces. Although breakdown due to microorganisms eventually occurred, later work with boric acid confirmed the preliminary results. Table 12 shows that in every case but one the peel

TABLE 13

WATER LOSS OF VALENCIA ORANGE HALVES, PEEL AND PULP, OBTAINED AUGUST 5, 1931; BUTTONS PARAFFINED

Half	Fresh weight, grams	Total loss of weight, grams				Dry weight as per cent of fresh weight
		1 day	3 days	6 days	9 days	
Orange County						
84 calyx	5,651	107	292	543	702	13.08
84 stylar	6,040	100	275	497	632	15.29
Riverside County						
100 calyx	7,097	141	389	701	907	12.71
100 stylar	7,745	127	350	616	775	14.87

of the calyx half of both citrus species permitted a greater water loss than that of the stylar half.

Sixteen fruits (table 6, group A), with calyx half of peel paraffined, lost 182 grams of water from August 20 to October 10, while the second lot of 16 fruits, with stylar half of peel paraffined lost 258.5 grams of water. In table 6, group B, the water losses were 199 and 359 grams, respectively. These results emphasize the greater water loss from the calyx half of Valencia oranges. Table 13 shows similar results with Valencia oranges from two sources, Riverside and Orange counties. The cut surfaces in this experiment were protected by boric acid powder. Table 13 also shows that the dry weight as a percentage of the fresh weight is less in the calyx halves than in the stylar halves, which means there is more water in the calyx half than in the stylar half. These results will be discussed more fully later.

Water losses from the two halves of lemons and Valencia oranges studied by the method of paraffining either half to the equatorial line are shown in tables 14 and 15.[5] Here again more water is lost through

[5] The water loss from the nonparaffined fruit surface (grams per square centimeter) may be calculated by dividing the water loss after any given period by the area of half of the surface of 16 fruits.

TABLE 14

WATER LOSS, WATER CONTENT, AND SURFACE AREA OF 16 VALENCIA ORANGES FROM RIVERSIDE*

Half paraffined†	Weight of fruit before being paraffined (grams)	Total loss of water from fruit, grams						Water content of fruit halves, grams		Area of surface of 16 fruits sq. cm.
		21.5 hrs.; rel. hum. 45; 84° F	116.0 hrs.; rel. hum. 46; 90° F	188.0 hrs.; rel. hum. 70‡; 80° F	284.0 hrs.; rel. hum. 86; 77° F	356.0 hrs.; rel. hum. 79; 75° F	452.0 hrs.; rel. hum. 68; 77° F	•Stylar	Calyx	
Stylar	4,619.0	29.0	161.0	240.0	276.5	306.5	363.0	1,919	1,965	2,362 (halves)
Calyx	4,871.5	19.5	114.0	174.5	201.5	225.5	270.0	2,025	2,072	2,421 (halves)

* Experiment started at 4 p.m., August 20, 1931.
† Cut-off stems paraffined in both cases.
‡ Rain.

TABLE 15

RELATIVE WATER LOSSES FROM CALYX AND STYLAR HALVES OF 100 TREE-RIPE LEMONS, FEBRUARY 3, 1932
(Paraffined Half Placed on a Collar on Plates of Glass; Button Paraffined)

	Weight of freshly picked fruit before being paraffined, grams	Water loss as per cent of original fresh weight									
		22 hrs.	49 hrs.	139 hrs.	188 hrs.	287 hrs.	353 hrs.	497 hrs.	549 hrs.	792 hrs.	1,050 hrs.
Evaporation from calyx half when stylar half of fruit is paraffined	10,010.50	0.80	1.94	3.96	5.05	6.75	7.87	10.60	12.18	17.34	21.82
Evaporation from stylar half when calyx half of fruit is paraffined	9,821.00	0.72	1.79	3.81	4.93	6.52	7.57	10.16	11.67	16.59	20 90

the peel of the calyx half than through that of the stylar half. The interrelations of the two halves must be considered when this method is employed. Any water lost from the paraffined half must make its exit through the uncovered peel of the opposite half. As has been shown, there are greater amounts of osmotically active compounds in the pulp of the stylar half than in that of the calyx half, which means there should be a greater resistance to movement of water from the stylar end toward the calyx end. It would be expected that this condition would have a retarding effect on water loss from the calyx half in fruit where the stylar half was paraffined. The fact that more water escaped from the calyx half indicates that the rind of that portion was more permeable to water and contained a smaller concentration of osmotically active substances (fig. 1) than the rind of the stylar half. Measurements not only of water loss but also of permeability and osmotic pressure confirm these assumptions.

It is noted from tables 14 and 15 that the differences in water losses from the polar halves of the fruit are much greater with Valencia oranges than with lemons. These differences parallel the relatively greater degree of sensitivity of the calyx region of the Valencia orange to certain rind affections. The differences are much less noticeable in the case of lemons.

Effects of Leafy Twigs on Water Losses During Wilting.—A better understanding of the significance of water gradients within the fruit may be obtained by studying the influence of leaves and twigs on the rate of water loss in fruits. Three lots, each consisting of 26 mature Valencia oranges, were picked August 18, 1931. Two groups were cut from the tree in the usual manner with fruit clippers, while the third group was cut so that an 18-inch twig bearing leaves was left attached to each fruit. The first lot was worked up at once, the fruit being peeled and the rind divided into calyx, stylar, and two middle quarters for comparison of water content and chemical composition. The pulp was divided into calyx and stylar halves, weighed, dried, and analyzed. The second and third lots were left on a table until September 1, 1931, when they were divided and treated as was the first group.

The water content of peel and pulp of these oranges when picked, and after 14 days in the laboratory, can be calculated from table 8. The losses from the peel were relatively larger than from the pulp.

There was considerable loss from the peel whether or not leafy twigs were attached, and the loss was slightly less in the case of attached leafy twigs. The greatest loss was from the calyx quarter and the least from the stylar quarter of the rind. The per cent of water in the calyx end of the peel was greatest and was found to decrease progressively to a mini-

mum at the stylar end. Conversely the stylar quarter of the peel had the most dry matter and the calyx quarter the least. Similarly the stylar half of the pulp contained a greater percentage of dry matter and less

TABLE 16

EFFECT OF GRADIENTS IN OSMOTIC PRESSURE ON THE WATER LOSS FROM FRUITS AT VARIOUS STAGES OF MATURITY

Maturity of fruit	Number of fruits	Treatment of fruit	After (days)	Water loss as per cent of original fresh weight
		Lemons		
Green	17	Control, debuttoned	0	0.00
	15	With shoots, but with leaves removed	3	2.25
	15	With shoots and leaves attached; leaves badly wilted after 3 days (dry weight of leaves, 57 grams)	3	5.12
Jumbo green	15	Control, debuttoned	0	0.00
	15	Buttons not sealed	4	1.83
	15	Buttons sealed with paraffin	4	0.79
	15	With shoots, but with leaves removed; shoots not sealed	4	3.75
	15	With shoots, but with leaves removed; shoots sealed	4	3.43
	13	With shoots not sealed; leaves attached and badly wilted after 4 days (dry weight of leaves, 60 grams)	4	4.49
	15	With shoots sealed; leaves attached and badly wilted after 4 days	4	4.13
Light green	14	Control, debuttoned	0	0.00
	14	With shoots, but with leaves removed	4	4.23
	12	With shoots and leaves attached	4	6.02
Silver stage	14	Control, debuttoned	0	0.00
	15	With shoots, but with leaves removed	3	4.80
	15	With shoots and leaves attached; (dry weight of leaves, 74 grams)	3	5.66
		Oranges		
Green Washington Navel	15	Control, debuttoned	0	0.00
	14	With shoots, but with leaves removed	3	4.72
	14	With shoots and leaves attached; leaves badly wilted after 3 days	3	9.85
Mature Valencia	32	Control, debuttoned	0	0.00
	30	With shoots, but with leaves removed	2	0.43
	31	With shoots and leaves attached; leaves badly wilted after 2 days (dry weight of leaves, 79 grams)	2	1.28

water than the calyx half. The pulp of the second and third groups had smaller percentages of dry matter than that of the first group. These reductions in dry matter may be due to material lost in respiration and volatilization.

Significance of Gradients in Osmotic Pressure.—The effect of changes in the maturity of the fruit in resisting water loss is explained, in part at least, by the reduced surface in relation to the volume of the fruit (Haas[12]) and by the increase in the osmotic pressure of the fruit cells, due to the storage of osmotically active solutes such as sugars and organic acids. For similar reasons mature leaves would have more power to withdraw water from fruit than immature leaves. Halma and Haas[15] have shown that the osmotic pressure of sap of leaves of a given age varies greatly throughout a single day. Seasonal changes in soil and air temperature were found by Haas and Halma[14] to affect the concentration of solutes in mature leaves. For periods of a given length of time under the same environmental conditions, mature lemon leaves lost more water per unit area than orange leaves. These water losses were directly related to the osmotic pressure of the leaf saps.

Table 16 shows a reduction of the gradient between the osmotic pressure of leaves and fruit as indicated by a lessening of the power of the leaves to pull water from the fruit as the fruit matures.

Figure 1 gives the approximate range of osmotic values of the sap of mature lemon and orange leaves. A comparison of these values with those for the peel and pulp of the calyx and stylar halves of 50 fruits indicates the possible effect of leaves in influencing water loss from fruit. Since the osmotic value of the sap of the stylar end of lemon peel is less than that of the calyx end, the former would tend to lose water more rapidly than the latter whether due to the withdrawal by the leaves or to direct evaporation from the fruit surface.

If the injury called endoxerosis or internal decline of lemon fruit is induced by a deficiency of water, the significance of the osmotic values of the various portions of the fruit becomes apparent. The first manifestation thus far discovered of the presence of this trouble in lemons is the appearance of pentosans and gums in the peel at the stylar end. Then follow the breakdown and drying of the pulp and core tissue with the accompanying pink to brown discoloration (Fawcett and Lee,[8] p. 422).

The invariable first appearance of the trouble at the stylar end may be accounted for in the following manner: While the osmotic value and sugar content of the pulp juice of the stylar portion are slightly greater than those of the calyx end, the osmotic value and sugar content in the peel of the stylar portion, where the internal decline begins, are lower than the values for the peel of the apparently unaffected calyx end. This indicates that the stylar peel would be the first to suffer from a water deficit.

The pulp of the stylar region, even though it has a slightly higher osmotic value than that of the calyx end, is the first portion of the pulp to be affected. A consideration of the anatomy of the fruit may make this understandable. Each juice vesicle of the pulp is attached to the periphery of the locular membrane just under the peel by means of a slender stalk or filament. Water is supplied from the vascular tissue of the peel. If that tissue is plugged with hydrophilic gum and pentosans, it is evident the supply of water to the underlying pulp is cut off and injury follows.

The influence of leaves and twigs on water loss from immature orange fruit was much more significant than in the case of mature fruit (table 16). This suggests the possibility that the injury called juice-sac granulation, which is most severe at the calyx end, may take place in immature fruit during periods of water deficit and become manifest as the fruit matures.

From figure 1 it is seen that the pulp of the calyx region has a smaller osmotic value than the remaining portions of the pulp and would therefore give up water more readily and be the first portion injured by a water deficit. The minimum osmotic value of the mature leaf sap is considerably greater than that of the pulp juice of the calyx end of the mature orange.

Diffusion from the Peel of Calyx and Stylar Regions.—Further information was gained from determinations on the amount of material which diffused inwardly when oranges were cut in halves and the pulp replaced with water.

Intact halves of orange peel were secured by removing the pulp from the halved fruit with a spoon. The peel was washed rapidly with distilled water and wiped dry. The stylar scar and button were sealed with paraffin and the halves with open end upward were supported on short pieces of mailing tube. The halves were nearly filled with distilled water and covered with watch glasses. At the end of several days the water was analyzed for dry matter; 3,400 p.p.m. was found in the water from the calyx halves and 6,500 p.p.m. in that from the stylar halves.

In a repetition of this method of measuring diffusion, tap water was used instead of distilled water. The fresh weight of 13 calyx halves was 553.5 grams, and the dry weight after diffusion had taken place was 80.25 grams or 14.5 per cent of the fresh weight. The fresh weight of 13 stylar halves was 583.5 grams, and the dry weight after diffusion had taken place was 86.25 grams or 14.8 per cent of the fresh weight. It has been shown that the per cent of dry matter in the fresh peel of the stylar half is greater than that of the calyx half. However, it is seen that after diffusion the values are nearly equal, being 14.5 and 14.8 per cent,

respectively, which means that more material was lost from the stylar than from the calyx half. Here the thin-walled cells of the white portion of the peel are in immediate contact with the solvent, while in later experiments on exosmosis from the intact fruit, the cutinized heavier-walled cells of the epidermis are in contact with the solvent.

The water loss through the depulped calyx and stylar halves of peel was determined when the halves were nearly filled with tap water. The halves were usually weighed individually in order to avoid the complete loss of the experiment as a result of a single leaky peel. Tap water was used instead of distilled water because it approaches being a balanced solution and therefore obviates much of the toxicity of distilled water. However, as will be shown, the difference between the concentration of tap water and orange juice is so great that toxic effects due to plasmolysis result. This was evident in an experiment in which the halves were filled with tap water. Thirty-eight stylar and 6 calyx halves showed greater water losses than the opposite halves of the same fruits.

As shown by further studies these results with tap water cannot be explained by a consideration of osmotic values alone. In whole oranges the peel is in contact with the pulp, the juice of which is extremely concentrated as compared with tap water. Moreover, the thinness of the stylar peel may contribute to the rapidity of the injurious effect of tap water. It is possible that under the conditions brought about by the contact with tap water, the stomata, which are closer together in the stylar peel, may, if not plugged, function more noticeably as pores in a septum because of the absence of concentrated orange juice. If instead of tap water we use a more concentrated solution such as orange juice in the halves, we approach more nearly the conditions as they exist in the orange.

Valencia orange juice was used instead of tap water to fill the peel halves in the following experiment. Where both halves contained the same composite juice of the whole pulp, there was a greater loss of water through the calyx peel, 26 calyx halves and only 1 stylar half showing greater water losses than the opposite halves of the same fruits.

The loss was also greater through the calyx peel when calyx-peel halves were filled with juice of calyx pulp and stylar-peel halves with juice of stylar pulp, the water losses from 17 calyx halves and only 1 stylar half exceeding those from the opposite halves. However, when calyx-peel halves were filled with juice of stylar pulp and stylar-peel halves with juice of calyx pulp, the loss was greater through the stylar peel.

These results emphasize the importance of osmotic concentration in the regulation of water loss from fruit. The greater concentration of

the juice from the stylar half of pulp caused less water to be lost through the peel, regardless of which half of the peel contained it. This minimizes the importance of stomata composition, and permeability of the peel as regulators of the water loss in fruit, and emphasizes the importance of osmotic concentration of the pulp juice.

Distribution of Stomata and Oil in Calyx and Stylar Peel of Valencia Oranges.—The distances separating stomata about a mother stoma were measured with stained and unstained cleared mounts. One hundred fourteen measurements at the stylar end showed the average distance between stomata to be 133.6 μ, while 81 measurements at the calyx end showed it to be 172.6 μ. Forty counts showed an average of 14.4 stomata per microscopic field at the calyx end and 17 at the stylar end. Thus the stomata about a mother cell appear to be closer together at the stylar than at the calyx end. From these results a greater water loss would be expected at the stylar than at the calyx end. Measurements of actual water loss are contrary to such expectations, and therefore indicate that water loss from stomata of citrus fruits is of minor importance when compared with the total water loss from the surface of fruits.

The larger amount of oil in the stylar peel may be a factor in decreasing the water loss from the stylar peel. However, as we have seen, the amount of oil in the peel and the number of stomata are only of secondary importance in comparison with the rôle played by the osmotic pressure of the juices of pulp, peel, and leaves in regulating water loss from fruit.

SUMMARY

The pH and buffer action of the juice of the calyx and stylar halves of mature Valencia orange pulp showed but small differences.

No significant differences were found in the specific gravity of the pulp juice of halves of either lemons or immature grapefruit. In both mature seedling grapefruit and mature Valencia oranges the specific gravity was least in the calyx and greatest in the stylar portion.

The carotinoid content of the juice of the stylar quarter of mature Valencia oranges was 1.54 times that of the juice of the calyx quarter. The juice of the calyx half of Valencia oranges had a greater iodine-fixing power than that of the stylar portion.

The total nitrogen in the juice of the stylar quarter of mature Valencia orange pulp slightly exceeded that in the juice of the calyx quarter. The amino nitrogen content of the juice of both quarters showed no differences. In the juice of Valencia and Washington Navel oranges the percentage of dry matter increased and that of total ash decreased from the calyx to the stylar end.

The pulp juice of mature Valencia oranges had a concentration of approximately 72,000 grams of total sugars in 1,000,000 cc at the calyx half and approximately 95,000 grams in 1,000,000 cc at the stylar half. The gradient in total sugar was so striking as to be easily recognized by taste. The pulp juice of the stylar halves of lemons and grapefruit contained more total sugars than that of the calyx halves, although the differences were not as large as those in Valencia oranges.

The pulp juice of the stylar halves of lemon had a slightly higher osmotic pressure than that of the calyx halves, while in Valencia orange the differences were much larger and in the same direction as in lemon.

Based on either fresh material or dry matter the calyx halves of Valencia oranges and grapefruit contain greater percentages of calcium and potassium and, in the ash, less phosphate than in the stylar halves.

A radial equatorial gradient was found in the ash of the pulp of Valencia oranges, the ash increasing toward the central core. Granulated pulp of Valencia oranges was found to have a higher percentage of water in the fresh material and higher percentages of ash, calcium, potassium, and sodium in the dry matter than that of control fruits.

The pulp of the calyx halves of mature grapefruit contained 10.87 per cent of total sugars in the dry matter while that of the stylar halves contained 13.12 per cent.

A greater amount of oil was found in the peel of the stylar end of mature Valencia oranges than in that of the calyx end.

The percentages of ash, calcium, magnesium, and iron in the dry matter of Valencia orange peel increased toward the calyx end, while those of sodium, potassium, and phosphate increased toward the stylar end.

A larger percentage of potassium was found in the dry matter of the gland-bearing portion of the peel of mature Valencia oranges than in the white portion, and a greater percentage in the gland-bearing portion of the stylar half than in that of the calyx half. In mature Valencia oranges a positive relation was found between phosphate and nitrogen, on the one hand, and sugar, but none between potassium and sugar. The percentages of potash in the gland-bearing portion of the peel of mature Valencia oranges from trees on potash-fertilized soil were higher than in fruit from trees on soil that received no potash.

The percentages of phosphate were greatest in the gland-bearing portion of mature Valencia orange peel. In this portion the percentages were higher for the stylar than for the calyx end. Valencia oranges from trees grown in sand cultures deficient in potassium showed breakdown of the peel.

The total sugars in the calyx halves of lemon peel (silver stage) were 14.59 per cent of the dry matter, while those of the stylar halves were 11.91 per cent. The calyx halves of grapefruit peel contained 28.77 per cent of total sugars in the dry matter while the stylar halves contained 27.89 per cent. In mature calyx halves of Valencia orange peel the dry matter contained 26.95 per cent total sugars while the stylar halves contained 23.17 per cent. In mature Valencia oranges the color reaction for starch appeared stronger in the stylar than in the calyx peel.

The osmotic pressure of the juice of the peel of the calyx halves of lemon (silver stage) was 15.08 atmospheres and that of the juice of the peel of the stylar halves 14.26 atmospheres. The corresponding values obtained for mature Valencia oranges were 20.00 and 24.58 atmospheres, respectively.

By measuring the rate of decolorization of $KMnO_4$ by materials that exosmose from fruit into water, small differences were found in the permeability of the halves of green lemons. The calyx halves of grapefruit and Valencia oranges had a greater permeability than the stylar halves. These and other differences may be related to certain diseases or physiological breakdowns.

The effect of hydrocyanic acid treatment of Valencia oranges was not only to increase the permeability of both halves but also to increase that of the calyx half more than that of the stylar half.

More water was lost through the peel of the calyx half than through that of the stylar half of lemons and Valencia oranges. The effect of leaves on the loss of water from fruits is considerably greater with immature than with mature fruits. The effect of increasing maturity of the fruit in resisting water loss is due to the increase in osmotic pressure that results from the storage of osmotically active solutes such as sugars and organic acids. The possible roles of osmotic pressure in certain physiological diseases of citrus fruit are described. The osmotic pressure of the juice of the calyx and stylar halves of pulp of mature Valencia oranges was able to regulate the water loss from the peel of detached oranges.

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